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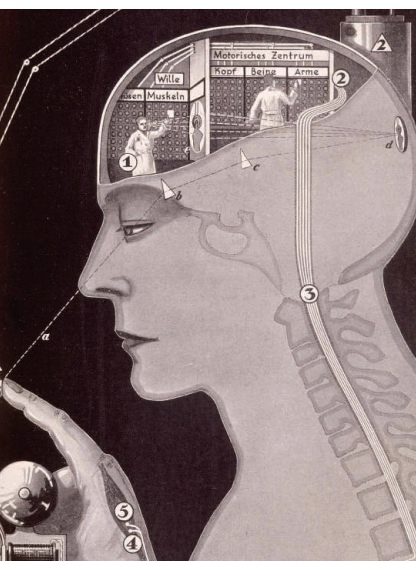
COVER

Male rhinoceros beetles (*Trypoxylus dichotomus*) wield a huge forked horn on their heads. Sexual selection may favor the evolution of exaggerated trait sizes because the developmental mechanism responsible for increased growth also results in signal reliability. See page 860.

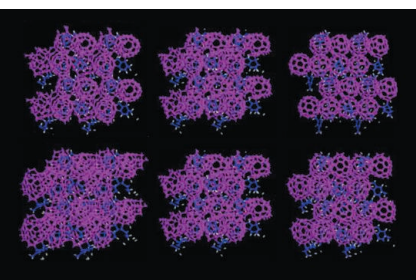
Photo: Will Freihofer and Douglas Emlen

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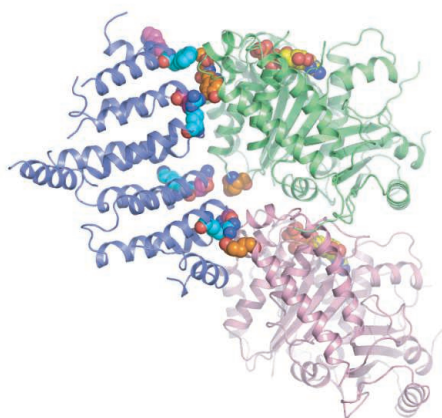
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Photo-tautomerization of Acetaldehyde to Vinyl Alcohol: A Potential Route to Tropospheric Acids

D. U. Andrews et al.

Enol tautomers may play a bigger role in atmospheric chemistry than previously suspected.

10.1126/science.1220712

The Precise Solar Shape and Its Variability

J. R. Kuhn et al.

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10.1126/science.1223231

Next-Generation Digital Information Storage in DNA

G. M. Church et al.

Digital information can be stored in DNA at densities higher than digital media such as flash memory or quantum holography.

10.1126/science.1226355

Intestinal Inflammation Targets Cancer-Inducing Activity of the Microbiota

J. C. Arthur et al.

Microbes resident in the gut can promote colorectal cancer in mice in an inflammation-independent manner.

10.1126/science.1224820

Single Reconstituted Neuronal SNARE Complexes Zipper in Three Distinct Stages

Y. Gao et al.

Zippering of a single SNARE complex generates high force and energy that can potentially drive synaptic membrane fusion.

10.1126/science.1224492

Predatory Fish Select for Coordinated Collective Motion in Virtual Prey

C. C. Ioannou et al.

Computer-generated prey evolve coordinated group behaviors when attacked by bluegill sunfish.

10.1126/science.1218919

SCIENCENOW

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Highlights From Our Daily News Coverage

Why Do Women Get More Migraines?

Brain differences may explain the disparity between the sexes.

http://scim.ag/Women_Migraines

Landscape of Dead Bodies May Have Inspired First Mummies

South American hunter-gatherers began mummifying their dead about 7000 years ago.

<http://scim.ag/First-Mummies>

Too Much Rock 'n' Roll?

A new technique could help designers create safer structures when fans rock too hard.

<http://scim.ag/Safer-Structures>

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The Signal Transduction Knowledge Environment

14 August issue: <http://scim.ag/ss081412>

RESEARCH ARTICLE: Interference with the PTEN-MAST2 Interaction by a Viral Protein Leads to Cellular Relocalization of PTEN

E. Terrien et al.

The G protein of rabies virus manipulates the cellular localization of PTEN, which may promote cell survival.

RESEARCH ARTICLE: Sequential Inter- and Intra-Subunit Rearrangements During Activation of Dimeric Metabotropic Glutamate Receptor 1

V. Hlavackova et al.

Both subunits in the metabotropic glutamate receptor 1 dimer move relative to each other before one adopts an active conformation.

PERSPECTIVE: L-Type Calcium Channels—On the Fast Track to Nuclear Signaling

M. D'Arco and A. C. Dolphin

In excitation-transcription coupling, some calcium signals are more efficient than others.

PODCAST

D. J. Emlen and A. M. VanHook

Growth of the male rhinoceros beetle's horn is more sensitive to insulin signaling than are other body parts.

>> Report p. 860

ST NETWORK: BioGRID

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ST NETWORK: ChEMBL

Access structural, chemical, and target information for bioactive compounds.

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15 August issue: <http://scim.ag/stm081512>

RESEARCH ARTICLE: A Paravascular Pathway Facilitates CSF Flow Through the Brain Parenchyma and the Clearance of Interstitial Solutes, Including Amyloid β

J. J. Liff et al.

FOCUS: Brain Superhighways

D. J. Begley

Cerebrospinal fluid flows through channels around brain blood vessels that are bounded by astrocytic endfeet, mediated by water transport through aquaporin-4.

RESEARCH ARTICLE: Targeted Tumor-Penetrating siRNA Nanocomplexes for Credentialing the Ovarian Cancer Oncogene *ID4*

Y. Ren et al.

FOCUS: Balancing Tissue and Tumor Formation in Regenerative Medicine

A. M. Bailey

Tumor-penetrating siRNA nanocomplexes credential *ID4* as a therapeutic oncogene target in human ovarian cancer.

RESEARCH ARTICLE: Elicitation of Broadly Neutralizing Influenza Antibodies in Animals with Prior Influenza Exposure

C.-J. Wei et al.

Broadly neutralizing antibodies to influenza can be elicited by vaccination in animals previously infected or immunized by the virus.

RESEARCH ARTICLE: Proteasome Inhibition Alleviates SNARE-Dependent Neurodegeneration

M. Sharma et al.

Neurodegeneration in a mouse model is abrogated by proteasome inhibitors that reverse impaired SNARE-complex assembly.

SCIENCE CAREERS

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Careers in Fast-Forward

M. Price

Winners of NIH's Early Independence Awards share the trials and successes of starting their own labs immediately after graduating.

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Tooling Up: Why Good People Leave Good Jobs

D. Jensen

Short stays do not look good to future employers—so why are they so common?

<http://scim.ag/GoodPeopleGoodJobs>

My Career, My Nonacademic Husband, and Me

A. Ambrosi

The implications of being in a mixed-career couple became apparent as lifestyle differences appeared.

<http://scim.ag/MixedDualCareers>

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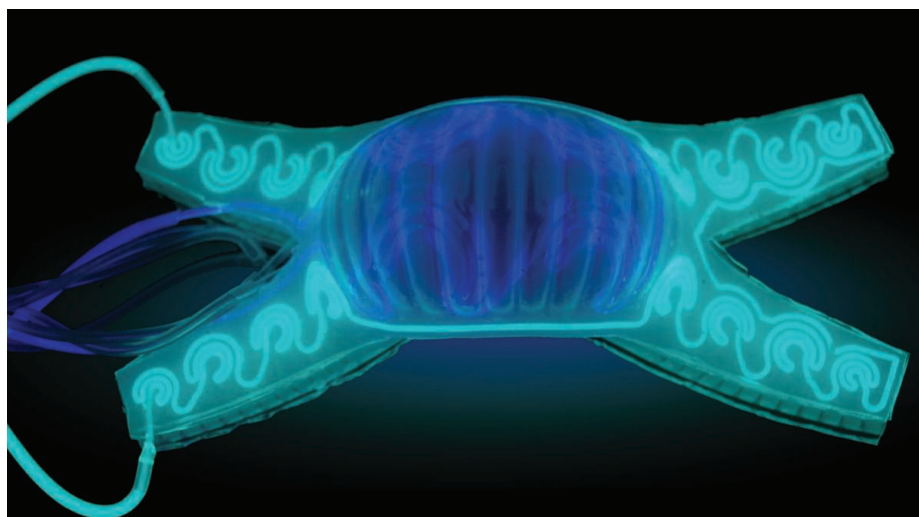
On the 17 August Science Podcast: citizens' roles in protecting species, biological evidence in courts, sequestration of the U.S. research budget, and more.

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ADVANCING SCIENCE, SERVING SOCIETY



Mechanical Chameleon

A wide range of animals can adapt their color patterns as a means of camouflage or otherwise changing their appearance. This is accomplished through changes in coloration, contrast, patterning, or shape. **Morin *et al.*** (p. 828) show at a basic level that some of these features can be added as microfluidic layers attached to mobile, flexible, soft machines. By pumping different fluids through the channels, the robots were able to change their coloration or overall contrast and could thus blend into the background of the surface they were lying upon. Conversely, by pumping through fluids of different temperature, the infrared profile of the robot could be changed without changing its visible coloration.

A State of High T_c Superconductivity

There are strong indications that high-temperature superconductivity in the cuprates is formed amid competing orders, but only two have been observed unambiguously. The so-called stripe order has been observed in a Lanthanum-based cuprate family and consists of coexisting charge-and-spin modulations and occurs at a characteristic dopant concentration in which the critical temperature T_c has a dip. Now, **Ghiringhelli *et al.*** (p. 821, published online 12 July; see the Perspective by **Tranquada**) have used resonant inelastic x-ray scattering to uncover a related but apparently two-dimensional charge order in the much cleaner YBCO cuprate family. The charge fluctuations were not commensurate with the lattice and did not originate in the characteristic oxygen chains of YBCO. The order appeared only in a narrow interval of dopant concentrations and competed with superconductivity, which provides a natural explanation for a plateau in T_c observed in the same range.

Amorphous Crystals

One usually thinks of a crystal as containing atoms, molecules, or other ordered units arranged in a periodic fashion to make a larger structure. **L. Wang *et al.*** (p. 825; see the Per-

spective by **D. Wang and Fernandez-Martinez**) compressed a solvate of xylene in C_{60} (fullerene) to very high pressures at room temperature, which caused the fullerene molecules to collapse and form amorphous clusters but with each cluster retaining its position in the original crystal lattice because of the interaction with the intercalated xylene molecules. Thus, a crystal was formed from amorphous constituents. On heating at atmospheric pressure, the xylene disappeared and the sample became completely amorphous.

Water In, Ozone Out

The danger of stratospheric ozone loss burst into public awareness in the 1980s, when the Antarctic ozone hole was discovered and described. Since then, the specter of ozone depletion in other locations, notably the Arctic, has been identified. Ozone loss is not confined to high latitudes, however, nor is it only the result of the addition of anthropogenic compounds containing chlorine and bromine in the stratosphere, as **Anderson *et al.*** (p. 835, published online 26 July; see the Perspective by **Ravishankara**) now demonstrate. Data from the atmosphere above the continental United States revealed that convective injection of water vapor into the stratosphere affects the free radical chemistry involving the (mostly anthropogenic) chlorine and bromine, thus accelerating ozone loss.

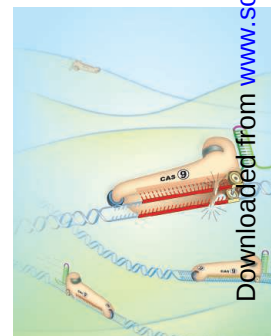
This process could become important in the stratospheric ozone budget if the frequency and intensity of these water-injection events, which are most common in the summer, increase as a result of global warming.

Synchronize Your Watches

In mammals, a central circadian clock in the suprachiasmatic nucleus (SCN) of the brain coordinates circadian changes in physiology and synchronizes other peripheral clocks. **T. A. Wang *et al.*** (p. 839, published online 2 August; see the Perspective by **Belle and Piggins**) report that daily changes in electrical activity of neurons in the SCN are coupled to the circadian clock through changes in metabolism—in particular, changes in reduction and oxidation state within the cell that reflect daily cycles of metabolism. The conductance of two kinds of potassium channels in the neurons was sensitive to redox state, thus linking the membrane potential and firing rate of the neurons to cycles of metabolic activity.

Ditching Invading DNA

Bacteria and archaea protect themselves from invasive foreign nucleic acids through an RNA-mediated adaptive immune system called CRISPR (clustered regularly interspaced short palindromic repeats)/CRISPR-associated (Cas). **Jinek *et al.*** (p. 816, published online 28 June; see the Perspective by **Brouns**) found that for the type II CRISPR/Cas system, the CRISPR RNA (crRNA) as well as the trans-activating crRNA—which is known to be involved in the pre-crRNA processing—were both required to direct the Cas9 endonuclease to cleave the invading target DNA. Furthermore, engineered RNA molecules were able to program the Cas9 endonuclease to cleave specific DNA sequences to generate double-stranded DNA breaks.



Take Another Cap

Selective cytosolic protein degradation is mediated primarily by the proteasome, which is comprised of a core peptidase and a regulatory cap. Current dogma holds that the 20S core peptidase of the proteasome functions exclusively with the 19S regulatory particle in eukaryotes, with PAN in archaea, and with Mpa in mycobacteria. **Barthelme and Sauer** (p. 843,

Continued on page 776

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This Week in Science

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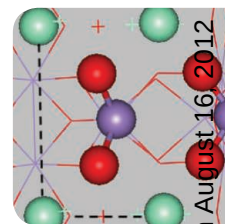
published online 26 July 2012; see the Perspective by **Matouschek and Finley**) now suggest that another type of proteasome can assemble in archaea consisting of the double-ring AAA+ protein, Cdc48, in complex with the 20S core protease.

Biology Made Me Do It

Introducing evidence of a biological basis of dysfunctional behavior might induce a judge to impose a lighter sentence upon defendants on the grounds that their innate biology lessened personal responsibility for their actions. On the other hand, it might also plausibly lead to a longer sentence on the grounds that this biological predisposition would imply a higher risk of future criminal acts. **Aspinwall et al.** (p. 846) analyzed sentencing opinions from a nationwide group of judges in the United States of a scenario closely modeled on a real-life case. Varying two factors—whether evidence for a biological basis was presented and whether it was offered by the defense or by the prosecution—revealed an overall lessening of sentences when biology was invoked.

Cleaning Diesel Exhaust

One strategy for removing pollutants from diesel engine exhaust is to trap the unburned carbon soot and then to combust the soot with the NO₂ that is generated from NO; the two pollutants are then converted to N₂ and CO₂. Diesel exhaust is relatively cold, compared to gasoline engine exhaust, and conversion of NO to NO₂ has required the use of platinum catalysts. **W. Wang et al.** (p. 832) now report that a more earth-abundant catalyst, based on Mn-mullite (Sm, Gd)Mn₂O₅ metal oxides was able to oxidize NO in simulated diesel exhaust at temperatures as low as 75°C. Spectroscopic studies and quantum chemical modeling suggested that Mn-nitrates formed on Mn-Mn dimer sites were the key intermediates responsible for NO₂ formation.



How to Make a Place Cell

Hippocampal place cells have been studied for more than 40 years, yet the mechanisms underlying their remarkable spatial tuning are still not established. Using whole-cell patch-clamp recordings in freely moving rats, **Lee et al.** (p. 849) changed the baseline membrane potential in hippocampal pyramidal neurons. Place fields arose spontaneously in otherwise silent pyramidal cells after depolarization when the membrane voltage reached a threshold. Place cells in the hippocampus and grid cells in the entorhinal cortex are thought to provide the framework for spatial navigation in an animal. However, it is still unclear how the hexagonal symmetry that is so prominent in grid cells emerges. While recording from the entorhinal cortex and in the pre- and parasubiculum during spatial behavior in the rat, **Krupic et al.** (p. 853) discovered that many forms of spatial periodicity exist in neurons in these structures. The grid cells seem to reflect a subset of this larger set, generated by self-organized dynamics.

All TOgether Now

$\alpha\beta$ -Tubulin is the polymerizing subunit of microtubules, which are dynamic polymers that have essential roles in cell division and intracellular organization. TOG domains are $\alpha\beta$ -tubulin binding modules that occur in the evolutionarily conserved Stu2p/XMAP215 family of proteins and promote microtubule elongation. **Ayaz et al.** (p. 857) used crystallographic and biochemical experiments to reveal that the TOG1 domain interacts with guanosine triphosphate-bound $\alpha\beta$ -tubulin in a conformation-selective manner, binding preferentially to a “curved,” microtubule-incompatible conformation. The binding mode apparently excludes analogous binding of a second TOG domain to the same heterodimer and may help to ensure polarized growth of microtubules.

Truthful Embellishments

Exaggerated ornaments such as beetle horns, deer antlers, and extreme tail lengths in birds are typically assumed to be subject to sexual selection because they signal the quality of an individual's breeding status—but how? **Emlen et al.** (p. 860, published online 26 July) present a general mechanistic model for the evolution of exaggerated traits, proposing that sensitivity to the insulin response pathway can explain variation among individuals. The exaggerated size of such ornaments and their increased variability between individuals are a result of sexual selection for traits that are honest signals of the fitness of the individual.

AAAS is here – promoting universal science literacy.

In 1985, AAAS founded Project 2061 with the goal of helping all Americans become literate in science, mathematics, and technology. With its landmark publications *Science for All Americans* and *Benchmarks for Science Literacy*, Project 2061 set out recommendations for what all students should know and be able to do in science, mathematics, and technology by the time they graduate from high school.

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Alan I. Leshner is the chief executive officer of the American Association for the Advancement of Science and executive publisher of *Science*.

Capably Communicating Science

THERE IS NO SHORTAGE OF TOPICS WHERE POLICY-MAKERS OR OTHER MEMBERS OF THE PUBLIC SEEM to persistently misunderstand, misrepresent, or disregard the underlying science: climate change, genetically modified foods, vaccines, or evolution, among others. Consequently, the call for scientists to do a better job of communicating both the meaning and the nature of their work is getting louder. Public understanding of science not only affects people's ability to appreciate and make full use of the products of science, it also contributes to the extent of support for scientific research. Yet far too many scientists are reluctant to engage with people outside their own community. The reasons range from a belief that this responsibility lies outside a scientist's "job description" to an expressed ignorance about how to go about it. In an attempt to find better solutions to this problem, the U.S. National Academy of Sciences convened a meeting of over 450 scientists, policy-makers, journalists, and other professional communicators to examine the underlying dynamics of science communication.* The good news is that empirical studies across many disciplines, particularly in the behavioral and social sciences, are providing very useful baseline information about public attitudes and knowledge about science, as well as some fundamental principles that can help guide scientists to engage more effectively with both the public and policy-makers.†

How does the public come to interpret and use science? Valuable studies have been carried out to discover what determines public attitudes toward science and technology, and some of the results are surprising. For example, some studies point to an individual's ideological views or cultural identity as having greater influence on his or her opinions than an understanding of the facts. Often, simply increasing public knowledge about an issue will not move the debate, as seen with embryonic stem cell research. Instead, the way an issue is framed can have a larger effect on people's views. As a case in point, many people will give more credit to the scientific claims about climate change when the issue is cast as a technological challenge than as a regulatory problem.‡

Science is complicated and often jargon-laden, so scientists may need help from a "translator" to help tell a story simply and cogently. In doing so, the gist of the message is what matters. Here there is a lesson to be learned from antiscience forces, who regularly oversimplify science in very effective ways, even when distorting it. As studies from psychology, sociology, and political science have shown, most people care primarily about things that affect them personally or locally; thus, a useful approach is to determine what matters to a specific audience and seek a way to make the message relevant to them. Although true public engagement attempts to bring together diverse perspectives, including the participants' political, personal, and community values, scientists are most effective when they stick to the facts. Because credibility is conferred by the audience, not the speaker, it is essential that scientists be seen as objective, citing the facts without an overlay of their own personal values.

Public understanding and support of science and technology have never been more important, but also never more tenuous. Today they are embedded in an increasingly politicized environment where ethical, legal, and social implications are emerging at a rate that seems to be outpacing society's capacity to make sense of the science. The science of science communication will be essential to help guide new and more effective efforts at engaging productively across the science/society interface.

— Alan I. Leshner

10.1126/science.1227898

*Arthur M. Sackler Colloquium, "The Science of Science Communication," National Academy of Sciences, Washington, DC, 21 to 22 May 2012 (<http://events.twworldwide.com/Events/NAS120521.aspx>).

†<http://communicatingscience.aaas.org/>. ‡D. M. Kahan et al., *Nat. Clim. Change* 10.1038/nclimate1547 (2012).



EDUCATION

The Cost of Improvement

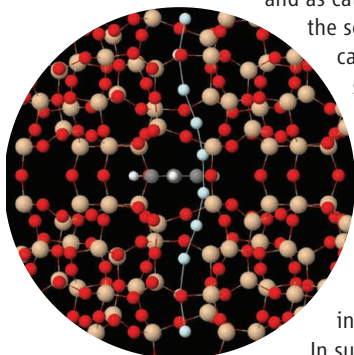
With increased emphasis on the role of science and technology in economic prosperity come increased efforts to improve science education. In the United States, science-focused education efforts occur on a backdrop of broader efforts to improve public education by using standardized tests of student achievement, largely limited to literacy and math. Because low test scores often come with steep consequences, the pressure to “teach to the test” can corrupt the system and undermine the very educational processes that are being monitored. Indeed, research has shown that high-stakes standardized tests focused on literacy and math in primary school can lead to decreases in the instructional time dedicated to other topics such as science. Maltese and Hochbein studied U.S. high schools in Indiana and found that despite school-level improvement of some schools on measures of math and literacy as reflected on a statewide standardized test used for evaluating schools (ISTEP), student-level performance in those improving schools did not demonstrate improvement in literacy or math on a separate, widely used college-entrance examination (ACT). Furthermore, school-level improvement on ISTEP math and literacy was generally associated with lower individual student-level science achievement on ACT. — BW

J. Res. Sci. Teach. **49**, 804 (2012).

MATERIALS SCIENCE

Blocking Zeolite Transport

Zeolites are microporous aluminum silicates, and because their pore and channel sizes can be similar to those of small organic molecules, they are used as adsorbents, in separations, and as catalysts. In some of the separation applications of zeolites, such as removing hydrogen from hydrocarbon gas streams, both large and small molecules occupy the material's internal channels.



In such processes, the larger molecules can partially block the channels and affect transport of the smaller molecules. Hedlund *et al.* measured the diffusion of helium in a ZSM-5 zeolite membrane in which they had adsorbed *n*-hexane or benzene. Loading with either

hydrocarbon could decrease the transport by more than two orders of magnitude. Percolation models with parameters determined from density functional theory, rather than simply fitting the results with adjustable parameters, described the measured mass transport of helium through the defect-free zeolite. For *n*-hexane, the mass transport of helium initially decreased gradually with hydrocarbon loading but then decreased abruptly at about 50% loading. For benzene, the decrease was observed at a lower hydrocarbon loading (19%). This stronger effect was caused by benzene adsorbing at the intersections of channels in the zeolite. — PDS

J. Membrane Sci. **415-416**, 10.1016/j.memsci.2012.05.009 (2012).

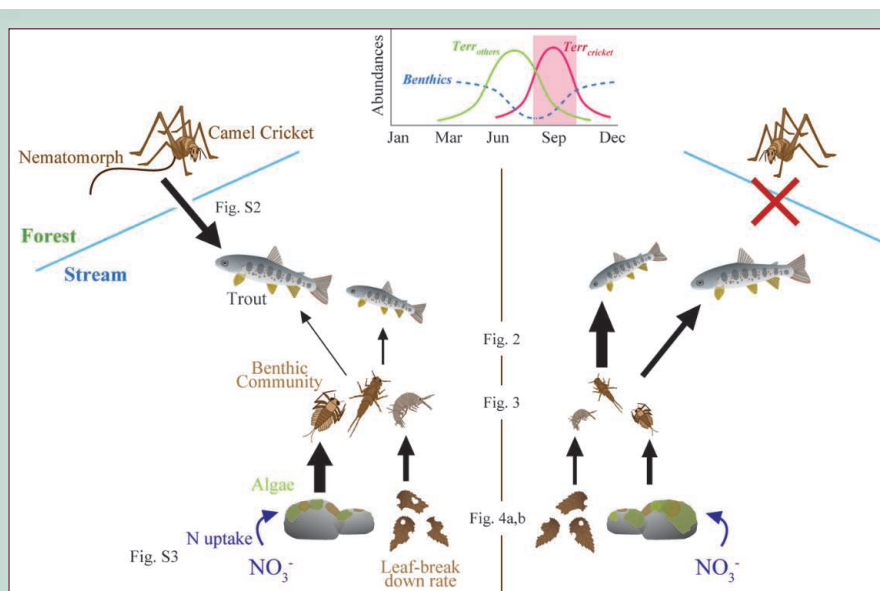
MOLECULAR BIOLOGY

Blunt End Protection

One of the problems with having linear chromosomes, as most eukaryotes have, is

their ends. Naked DNA ends are recognized by the cell as breaks in the continuity of the genome and are repaired with all haste. Trying to “repair” the ends of chromosomes, however, could have devastating consequences for the cell (the fusion of whole chromosomes, for example). Instead, special structures at chromosome ends, known as telomeres, whose general features have been conserved across evolution, protect them from the attentions of the cell's DNA repair machinery. Telomeres generally have a single-stranded (ss)DNA overhang that binds to proteins that help disguise the DNA end.

Surprisingly, Kazda *et al.* find that a substantial fraction of the telomeres in the plant *Arabidopsis thaliana* do not have canonical overhanging ssDNA ends. Rather, the chromosomes have blunt ends, which are probably a consequence of the replication of DNA. These blunt ends, which would be unable to bind the protective protein complex found on the other *Arabidopsis* telomeres, are bound by



ECOLOGY

A Cascade of Consequences

Specialized interactions between pairs of species can have wide and surprising consequences for the wider ecosystem they inhabit. Sato *et al.* investigated the case of the nematomorph parasitic worm, which has a life cycle involving a free-living adult stage and several parasitic larval stages, the last of which parasitizes cricket hosts. The relationship is manipulative, in that the worm induces the cricket to head for stream waters, where the adult worm will emerge to complete its free-living reproductive phase. The authors show experimentally that the addition of crickets to a Japanese stream ecosystem diverts predatory trout towards this attractive source of food and away from their normal diet of benthic invertebrates, in turn leading to a decrease in benthic algae and an increase in the rate of leaf-litter breakdown. Because of the ubiquity of nematomorphs at streams and streambanks globally, the cascading effects of their manipulative parasitism may be a common feature of these ecosystems. — AMS

Ecol. Lett. **15**, 786 (2012).

the Ku70/80 heterodimer, which is normally required for DNA repair but can also act to protect free DNA ends. — GR

Genes Dev. **26**, 1703 (2012).

GENETICS

Commensal Complexity

Strains of the *Pseudomonas fluorescens* group of commensal bacteria offer plants protection against pathogens through the production of antibiotics or the stimulation of plant resistance. The specific protections offered vary between strains, suggesting that genetic variation may underlie these differences. In order to investigate this, Loper *et al.* sequenced seven plant-associated strains that differed with respect to species, location on the plant, and mechanism of protection. A comparison of these genomes to three previously sequenced strains showed that whereas approximately 50% of the genes overlapped among all sequenced *P. fluorescens* strains, forming a core genome, the remaining genomic properties were highly variable and could be used to distinguish three pan-genomic complexes, with a high number of strain-specific elements and genes. Within the clades, specific genes and genetic elements tended to be found only within a select subset or single strains, some of which underlie phenotypic characteristics and may be involved in multitrophic interactions such as the production of secondary metabolites and components of the type III secretion system. The tremendous genetic diversity observed among these strains highlights how such investigations can help us better understand the complexities of species interactions. — LMZ

PLoS Genet. **8**, e1002784 (2012).

CHEMISTRY

Angle of Attack

In qualitative terms, chemists have become adept at predicting how reaction probabilities depend on the relative dispositions of two colliding reagents. For instance, if a reactive site is bounded by a large fragment and a small one, the collision partner is more likely to make it past the small one. This steric effect is the basis for much of asymmetric catalysis. A more detailed, quantitative picture of what happens at any particular collision angle is rather harder to capture. Wang *et al.* have now mapped out the precise three-dimensional steric constraints guiding an elementary chemical reaction: chlorine's abstraction of a hydrogen atom in the vibrationally excited C-H bond of CHD₃ to produce HCl. By varying the polarization of the vibrational

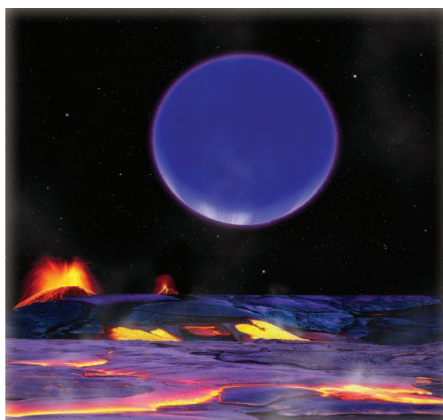
excitation laser, the authors could effectively choose the alignment of the (partially deuterated) methane reagent relative to the incoming Cl atom beam, after which they used ion imaging to map out the product trajectories and their associated quantum states. The results for the production of vibrationally relaxed HCl are largely in keeping with expectations from the longstanding line-of-centers model in collision theory, whereas pathways leading to vibrationally excited HCl manifest more complex dynamics. — JSY

Nat. Chem. **4**, 636 (2012).

ASTROPHYSICS

Chaotic Planets

It may not seem so, but the orbits of the planets in our solar system are chaotic, meaning that they are unpredictable beyond a characteristic time scale, known as the Lyapunov time, which in the case of the solar system's inner planets is about 5 million years. Deck *et al.* now show that the orbits of the two planets in the Kepler-36 system are chaotic, with a Lyapunov time of less



than 10 years. The orbits of Saturn's moons Prometheus and Pandora are the only other ones known to show chaos on such a humanly observable time scale.

The two planets orbit a distant star, which has a mass similar to that of the Sun and is among the 156,000 stars regularly monitored by NASA's Kepler space telescope. The planets have masses around 4 and 8 times that of Earth and orbit so close to one another that the angular size of the heavier planet as seen from the lighter planet would be 2.5 times as large as the full Moon viewed from Earth (see Carter *et al.*, Reports, 3 August 2012, p. 556; published online 21 June 2012). The authors suggest that the orbital chaos is a consequence of mutual gravitational interactions between the two planets. — MJC

Astrophys. J. **755**, L21 (2012).

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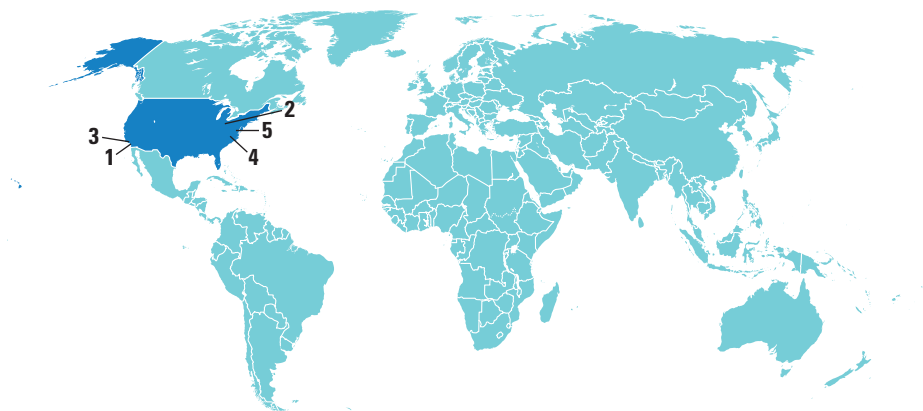
things you didn't
(and 3 you probably
shouldn't) know
about some of
your most
respected
colleagues.

One more data point on why
you should spend more time
at membercentral.aaas.org.
There you can enjoy a feast
of blogs, videos, webinars,
discounts, and downloads
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membercentral.aaas.org

AROUND THE WORLD



Pasadena, California 1

Curiosity Takes a Look Around

Three days after landing on Mars, Curiosity sent its first color, 360-degree look at Gale Crater (above) to scientists at NASA's Jet Propulsion Laboratory in Pasadena, California, late in the day on 8 August. The panorama, composed of 130 thumbnail images taken with the rover's Mast Camera, shows some targets for future exploration: gray patches in the foreground, where rocket engines blasted the ground during the rover's descent, and in the distance, reddish-brown dunes cut across by a gray layer. Over the next 2 years, Curiosity will cross the rocky surface of the crater on its way to Mount Sharp, the crater's interior mountain.

Indiana and Ohio 2

Swine Flu Virus in Midwest

An unusual influenza virus spreading from pigs to children has caught the attention of public health officials. The virus, H3N2v, first surfaced in July 2011 and seasonally infects humans but has not caused severe disease. According to the 10 August issue of the *Morbidity and Mortality Report*, which is published by the U.S. Centers for Disease

Control and Prevention (CDC), the country has had 153 cases since 12 July.

More than 90% of the H3N2v cases occurred in children, most of whom had direct or indirect contact with pigs, and all but two were in Indiana and Ohio. Researchers are intrigued because H3N2v has an internal "M" gene that matches one found in H1N1; this M gene may play a role in the current transmissions, by giving it an advantage for circulating in pigs, says CDC virologist Michael Shaw. "And if it becomes more common in pigs, it gives more of a chance to jump into humans," Shaw says. However, there's no evidence that this M gene alone leads to human-to-human transmission.



Studies have shown that the current flu vaccine likely will not protect against this strain, but adults may have immunity from exposure to "wild type" strains of influenza as well as previous vaccines.

<http://scim.ag/swineflu2>

Thousand Oaks, California 3

Amgen Pulls Cancer Drug

The failure of a pancreatic cancer therapy, announced last week by its maker Amgen, is another sign that a once-promising class of cancer treatments that target a particular hormone isn't helping as hoped. The latest therapy to flop, called ganitumab, targets type 1 insulin-like growth factor receptor (IGF-1R). After a phase III clinical trial of the therapy, the company concluded it wouldn't help patients live longer and pulled the plug.

IGF-1R was deemed a promising target after researchers found that higher levels in a person's blood were linked to a slightly greater risk of cancer. Overexpressing the hormone in the lab seemed to cause tumors in cells and animals that shrank when treated with an IGF-1R blocker.

But in patients, the drugs are "a serial failure," says Vuk Stambolic, a cancer researcher at Princess Margaret Hospital and the Ontario Cancer Institute in Toronto, Canada. One problem is that it may not be enough to go after IGF-1R alone, especially in patients with very advanced disease. The work in animals and cells also may not fully mimic real life. "I think that the model is probably only partially representative of what happens in cancer," Stambolic says.

<http://scim.ag/IGF1Rpulled>

Raleigh 4

U.S. Public Wary of GM Mosquitoes

Most people oppose using genetically engineered male mosquitoes to control wild mosquito populations—when they learn the potential risks, a survey released on 8 August by researchers from North Carolina State University suggests. The mosquitoes have been released in other countries to control mosquito-borne diseases, but have not yet been used in the United States. The survey, which polled 1211 people, is the first to gauge U.S. public opinion on the technology.

People were most receptive to the idea when the mosquitoes were described as "sterile," rather than "genetically modified" or "transgenic," says political scientist

The Universe in 3D

Researchers with the Sloan Digital Sky Survey III last week released the largest three-dimensional map of black holes and massive galaxies yet produced, which pinpoints the locations and distances to more than 1 million galaxies (<http://www.sdss3.org/press/dr9.php>). Mapping these galaxies will enable scientists to retrace the history of the universe for the last 6 billion years, the team says, thereby allowing astronomers to make better estimates for how much of it is composed of the “dark matter” that can’t be directly seen, and “dark energy,” the mysterious force that’s driving the expansion of the universe. The survey, which recently completed its second year, has so far covered about 8% of the sky. By the time the 6-year project is completed, researchers will have mapped all massive galaxies outside of the dust-clogged plane of our Milky Way galaxy that are visible from the Northern Hemisphere—altogether, about one-fourth of the sky that’s visible from Earth.

Michael Cobb, who supervised the survey. But using the word “sterile” in public information campaigns could be misleading, he says: When genetically engineered male mosquitoes breed with wild females, they pass on a defective gene which causes their offspring to die early.

Approval increased when people learned more technical information, but when potential risks were explained—such as the possibility that the altered genes in any surviving female offspring might cause a new allergy, or that a more noxious animal might fill the mosquito’s ecological niche—approval plummeted to 33% for “sterile” and 17% for “genetically engineered” mosquitoes.

Washington, D.C. 5

To Our Star and Beyond While Staying Within a Budget

The latest pitch for doing science in space takes a decidedly budget-wise approach. Released on 15 August, the report by the National Research Council (NRC) of the U.S. National Academies lays out a wish list from the heliophysics community. It calls for exploration in the next decade of what drives solar activity and how that activity affects the solar system, especially Earth and its inhabitants. And most of it would be done within the slowly increasing \$650-million-per-year budget specified by NASA for heliophysics.

A key to the decadal plan is a redefinition of medium-size heliophysics space missions. Three proposed missions would be conceived by the community, led by

a principal investigator, and constrained to a cost of \$520 million each. “This is the single best way we’ve found to contain costs,” says the committee’s chair, Daniel Baker of the University of Colorado, Boulder.

Not that heliophysics has no grand ambitions: A recommended billion-dollar mission later in the decade—a constellation of spacecraft to investigate how Earth’s atmosphere absorbs solar wind energy—would require a budget boost if it is to be in service by the next solar maximum in 2024.

NEWSMAKERS

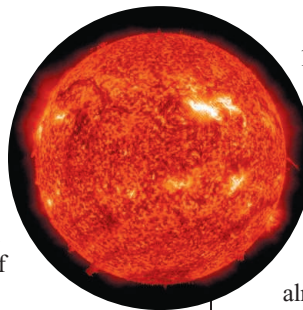
Founder of Jodrell Bank Observatory Dies

Physicist and radio astronomer **Bernard Lovell**, who was the founder and first director of the Jodrell Bank Observatory in Cheshire, U.K., died on 6 August. He was 98.



Lovell

Lovell headed the observatory from 1957 to 1981. He conceived the idea for the observatory’s 76-meter-diameter Lovell telescope in 1945; it became the world’s largest telescope upon its completion in



1957, and it was also the only telescope in the West able to track the Soviet Union’s Sputnik 1 satellite.

Under Lovell, teams at the observatory made important discoveries: The telescope was the first to spy quasars, and Jodrell Bank scientists have discovered almost two-thirds of the known pulsars.

FINDINGS

Compound Points Toward The Pill for Men

A contraceptive pill for men has stumped researchers for decades. But new research published in *Cell* this week suggests there may be hope. A small molecule given to male mice renders them infertile without affecting the animals’ testosterone or other hormone levels. And the effect is reversible: When the researchers stopped giving the animals the compound, their sperm production came back and they were able to sire healthy offspring after 1 or 2 months.

The compound, called JQ1, blocks the function of a protein called BRDT, which is crucial for sperm development. It was originally developed as an anticancer agent, targeting a protein related to BRDT called BRD4. Although the mice in the study seemed to have no obvious side effects while on the treatment, study authors Martin Matzuk of Baylor College of Medicine in Houston, Texas, and James Bradner of Harvard Medical School in Boston say that a more specific compound, which targets only the testis-specific >>

Random Sample

Family Matters

Look closely: Each intersection of warp and weft in this blanket represents a single point of genetic data. When taken as a whole, the blanket displays an entire genome—that of the sister of bioinformatician Manuel Corpas of the Genome Analysis Centre in Norfolk, U.K.

On 20 June, Corpas began a crowdfunding effort, hoping to raise \$20,000 to sequence his own and his family's entire genomes. The project, he says, was part of his ongoing effort to raise awareness of a coming revolution in personal genomics. "My vision is about accessibility," he says. "Anyone in the world who would like to know about their genetic data should have the resources to learn about it." As a part of that, he plans to develop free software that can help people interpret their own genomes.

After raising only one-fifth of the money he hoped for, last week Corpas called off the funding campaign—but he hasn't given up his plans to raise awareness. He had already sequenced and published 1 million base pairs for each of his participating relatives through the genetic testing company 23andMe, and thanks to his crowdfunding effort, he says he's now raised enough to sequence the exomes—protein-coding sequences of DNA—of his sister and his parents.

Textile artist Ben Landau, who created the blanket, is among those who have uploaded the Corpas family's free genetic data. In an age when parents may soon be able to alter the genes of their offspring, Landau says his blanket is meant to "highlight the sanctity" of our unique individual genomes. <http://scim.ag/corpasgen>



BY THE NUMBERS

799 meters Record depth in the Gulf of Mexico for the coral *Lophelia pertusa*, found on energy platforms by a team of federal and university scientists.

£15.3 million Amount of funding, announced on 14 August, for the Royal Society and the U.K. Department for International Development's Africa Capacity Building Initiative to support research in sub-Saharan Africa.

877 times higher The extinction rate for freshwater fish species in North America from 1900–2010, compared with the rate found in the fossil record (one species per 3 million years), according to U.S. Geological Survey scientists reporting in *BioScience*.

artisanal fishing, tourism, and other coastal livelihoods. "The index shifts our focus to what people want from oceans and how we achieve that in a sustainable way," says lead author Benjamin Halpern of the University of California, Santa Barbara. The team gathered global data from about 171 countries and ter-



ritories, set performance targets, and scored the countries for current conditions and trends. Scores ranged from 36 (Sierra Leone) to 86 (uninhabited Jarvis Island); only 5% of locations scored higher than 70. The holistic index is "a breakthrough on a number of counts," says Margaret Caldwell of Stanford University, who was not involved. <http://scim.ag/oceanindex>

>>FINDINGS

BRDT, is needed before testing in healthy men. That should be possible, they say; colleagues at the University of Oxford have identified the detailed crystal structures of BRDT interacting with JQ1, which provides helpful clues. <http://scim.ag/manpill>

Written in (DNA) Code

As the amount of digital information grows at an ever faster pace, the race is on to find new storage media. A paper published online 16 August in *Science* offers one surprising entrant: DNA.

Less than a gram of DNA can store hundreds of petabytes (millions of gigabytes) of information. But you need a practical system for reading and writing. So a team led by George Church of Harvard Medical School in Boston created a code for converting a digital file into short DNA fragments. Each fragment carries a "barcode" address that records its location in the original file, and a chunk of digital data, coded

as a string of zeroes (A or C in the DNA alphabet) and ones (G or T).

To test the code, the team converted a genetics book co-written by Church into DNA fragments, and then printed them onto a DNA chip. After resequencing the DNA fragments from the chip and converting them back into the book, the error rate was only about 2 bits per million—comparable to a typical DVD disk, but far smaller. <http://scim.ag/DNAcodes>

Ocean Health Index Shows Room for Improvement

An ambitious new scorecard aims to give the broadest view yet of the condition of the world's oceans and their ability to provide benefits to humans. Overall, the oceans score 60 out of a possible 100 points, which the authors describe in this week's issue of *Nature* as "a cautionary message."

Rather than focusing on only marine life conservation, the new index also considers human uses of the oceans, such as

PUBLIC HEALTH

Policy Shifts on Emergency Use of Cholera Vaccines

Fighting without vaccines.
Cholera patients in a treatment center
in Port-au-Prince in November 2010.

Nobody disputes that cholera can be a fast and vicious killer. But whether vaccination can do much to stop an outbreak once it has begun has been vigorously debated for years. Many experts have argued that in outbreak situations—especially in the poor, messy places where cholera often strikes—existing vaccines are too expensive, not effective enough, and too impractical to roll out; they might even make matters worse, some fear, because they distract health workers from treating patients or improving water and sanitation, the cornerstones of cholera control.

But now, the tide appears to have turned. This week, a technical working group at the World Health Organization (WHO) is set to publish a report advocating for the creation of a global stockpile of cholera vaccines that would be rushed to countries when an outbreak begins. WHO has already adopted the recommendations, and if donors can be persuaded to come up with the money, some \$10 million per year, the stockpile could be established in 2013, setting the stage for a much broader use of cholera vaccines.

The report reflects a gradual change of opinion that may also have a big impact in Haiti. After a devastating cholera outbreak erupted in 2010, the Haitian government, advised by the Pan American Health Organization (PAHO), decided against vaccination. But in June, Partners In Health (PIH), a Boston-based charity, completed what it says was a successful 3-month pilot campaign, set up with the government's blessing, to vaccinate 100,000 people. The program showed that vaccination is feasible even in very difficult circumstances, the group says. After *Science* went to press this

week, an expert group at PAHO was scheduled to discuss the campaign; PAHO Deputy Director Jon Andrus said on Monday that he was "very confident" that the group will advise the Haitian government to start expanding vaccination.

"I have the feeling that the world is coming around on this issue," says John Clemens, who, as director of the International Vaccine Institute in Seoul, helped develop Shanchol, the cholera vaccine used in Haiti. (Shanchol is produced by Shantha, an Indian subsidiary of vaccinemaker Sanofi Pasteur.) Clemens, now at the University of California, Los Angeles, has long been frustrated by the resistance to cholera vaccines; a paper he co-authored last year argued that in the 2008–2009 cholera outbreak in Zimbabwe, vaccination could have saved almost 1700 of the more than 4200 patients who died.

But at the time, WHO and some aid organizations said Zimbabwe was in no position to run a cholera vaccination campaign; its weak health care system was already overburdened by trying to contain the outbreak and treat patients. The fact that cholera vaccines come in two doses given 1 to 2 weeks apart was seen as another obstacle, because it can be hard to make sure people get their second dose. Vaccination might also give people a false sense of safety, opponents said, because the existing vaccines are only between 60% and 85% effective. Another concern was that vaccines would divert attention from the most important way to prevent cholera and many other waterborne diseases: keeping drinking water and human feces separate.

At the start of the outbreak in Haiti, simi-

lar discussions split the scientific and public health communities. The shortage of vaccine was another argument against vaccination; the Haitian government worried that vaccinating a small part of the population could spark discontent and social unrest. As a result, vaccination was left out of the package of control measures.

But as the number of cases mounted in Haiti, the issue soared to prominence on international political agendas. In 2011, the World Health Assembly, an annual meeting of health ministers in Geneva, adopted a resolution calling for more effective cholera control, including the use of vaccines "where appropriate." After further consultations, WHO asked the technical working group to come up with an implementation plan for a global stockpile.

WHO's approval of Shanchol for use in 2011 also helped bring about the change. At about \$1.85 per dose, Shanchol is cheap compared with its main rival, Dukoral, made by Dutch biotech Crucell and mostly used as a travelers' vaccine. It's also easier to ship and distribute, because unlike Dukoral, it doesn't need to be co-administered with 150 milliliters of a buffer solution.

Clemens says perhaps the clearest sign that views on vaccination are changing is that the Swiss arm of Doctors Without Borders (MSF)—a group that has long had reservations about the introduction of cholera shots—responded to an outbreak in the West African nation of Guinea by vaccinating about 200,000 people this year. Iza Ciglenecki, an epidemiologist at MSF Switzerland, says standard control methods—which include finding patients and treating them with a solution of sugar and salt to

keep them hydrated—can drive down mortality but don't have much impact on the number of cases; vaccination does.

In the two coastal regions of Guinea where the campaign was carried out, 75% of the population received two shots, she says. "Maybe that doesn't sound fabulous, but this area has a very mobile population," making it harder to catch people twice.

In Haiti, PIH and a local NGO called GHESKIO started their pilot program in April after many delays. It involved 50,000 people in a slum in the capital Port-au-Prince, says PIH's Louise Ivers, and another 50,000 in a rural area in the Artibonite valley. More than 90% of people came for their second shot in both places. "We've shown that the problems are not insurmountable if you're very dedicated," Ivers says. There's no evidence that the vaccine led people to abandon precautionary measures such as washing hands and purifying water, and PIH has brought up the importance of clean water and sanitation in every discussion about the program, she adds, to convey the message that vaccines are no excuse for neglecting infrastructure.

Even for countries that want to use vaccines to stem the spread of a cholera epidemic, availability has long been an issue. Currently, the two vaccine manufacturers have very limited amounts of the vaccine on the shelf, and they cannot easily ramp up production when demand surges. That's where the plan for a global stockpile comes in. It's modeled on similar caches of meningitis and yellow fever vaccines, and it would be run by the International Coordinating Group, a quartet consisting of MSF, WHO, the U.N. Children's Fund, and the Red Cross, which also controls those stockpiles.

Under the plan, countries that have a confirmed cholera outbreak can ask for vaccines to be shipped; a special committee will have to decide within 48 hours. It can refuse if a vaccine isn't going to make much difference, says WHO scientist and working group member Alejandro Costa, for instance, because the outbreak is unlikely to become big, or it has already peaked. Governments will also have to produce a feasible strategy to carry out a campaign.

The working group suggests that the European Union, the Bill & Melinda Gates Foundation, the GAVI Alliance, and the U.K. and U.S. governments fund the stockpile; Costa says WHO is already talking to them, and some are interested. "This report is a big jump," he says. "We are moving fast with this."

—MARTIN ENSERINK



Three's company? LSST hopes to join two other telescopes on this mountaintop in northern Chile.

2013 U.S. BUDGET

Big or Small, Science Will Suffer If Sequestration Goes Into Effect

J. Anthony "Tony" Tyson, director of the proposed Large Synoptic Survey Telescope (LSST), felt last month like he had won the lottery. After a decade of tough negotiations with the U.S. National Science Foundation (NSF), the agency's oversight body said the \$665 million project was ready to be built. But in the months to come, Tyson, an astrophysicist at the University of California, Davis, may discover that getting a green light to seek \$465 million from NSF's large facilities account is more akin to winning permission to book passage aboard the *Titanic*.

For U.S. scientists doing research supported by the federal government, the 2013 fiscal year, which begins on 1 October, could be catastrophic. The financial iceberg heading their way even has a name: sequestration.

The word comes from the Budget Control Act, passed in August 2011 to avert a federal default. The law created a congressional commission, which last fall came up with a long-term strategy to reduce the country's \$1.5 trillion annual deficit.

But Congress failed to adopt the commission's recommendations. Its inaction triggered the second component of the act: a decade of mandatory spending cuts totaling \$1.2 trillion. The first bite—\$110 billion

across all agencies, divided equally between military and civilian programs—is scheduled to take effect on 2 January.

It may be the most unpopular law in Washington. All the key players in last summer's high-stakes negotiations—President Barack Obama, Senate Majority Leader Harry Reid (D-NV), and Speaker of the House of Representatives John Boehner (R-OH)—have repudiated its blunt approach to deficit reduction. In particular, Republicans have tried to fence off military spending, while Democrats want to protect

domestic programs. White House budget director Jeffrey Zients says that sequestration "is bad policy, was never meant to be implemented, and should be avoided,"

Online

sciencemag.org

Podcast interview with author Jeffrey Mervis (http://scim.ag/pod_6096).

and this month he and another senior Administration official told Congress that the law was "highly destructive" and would lead to "senseless chaos." In fact, regret runs so deep that many believe Congress will find a way to postpone or avoid the cuts it mandated.

The uncertainty has left federal agencies groping in the dark. The Administration has

promised to follow the terms of the Budget Act. But on 31 July, Zients told each agency to “continue normal spending and operations since more than 5 months remain for Congress to act.” In reality, meaningful negotiations aren’t likely to begin until after the 6 November national elections, when legislators return for a lame-duck session. Another complication is that the cuts would apply to a FY 2013 budget that Congress is not likely to approve before next spring (*Science*, 10 August, p. 631).

Last week, Obama signed a measure, embraced overwhelmingly by legislators from both parties, that requires him to spell out the consequences of sequestration for each agency within 30 days. But Administration officials have already said the overall impact on science would be “devastating,” translating into an immediate cut of 8% to 10% for every federal research agency.

For two bastions of federally funded basic research, NSF and the National Institutes of Health, a cut of that size would mean making thousands fewer awards than planned and possibly trimming the size of current grants. Graduate students would need to find other sources of funding, and efforts to improve science and math education at all levels would suffer. In addition, support for innumerable programs, initiatives, and activities around the world would be curtailed or canceled.

The White House is hoping that the scientific community will help persuade Congress to reject that meat-ax approach. Last week, White House officials gave a pep talk to university and scientific society administrators, assuring them that the president opposes any further cuts to programs that fund research and science education.

A better way to eliminate the deficit without decimating vital government activities, Zients says, is “bipartisan, balanced deficit legislation.” That’s shorthand for a mixture of tax increases and spending cuts. But raising taxes is anathema to Republicans, and many Democrats object to any large cuts to domestic programs.

One scientist who participated in last

week’s briefing doubts that the White House will be any more specific next month. “When the other side won’t say what they will cut, you don’t want to make enemies by spelling out the details,” says Gilbert Omenn, a professor of medicine at the University of Michigan, Ann Arbor, who served as both a budget and a science officer under former President Jimmy Carter. Omenn’s biggest fear is that Congress will exempt military spending, placing an even heavier burden on civilian programs. But he thinks Congress is more likely to simply push back the start date for sequestration. In the meantime, science agencies are warning their constituents to prepare for the worst.

Yet Tyson remains optimistic. One reason is his unshakeable faith in the value of LSST. The telescope will teach scien-

end of the agreement. (Private donors have already put up the remaining \$40 million.) If not, he says, “other things will intrude and we won’t be able to stay on schedule.” And a delay would inevitably increase its overall construction cost.

Sequestration poses a serious threat to that schedule, however. At a minimum, NSF would need enough money in its large facilities account to build LSST without squeezing other projects already in the queue. NSF requested \$197 million for the account in 2013 to continue work on four projects—the National Ecological Observatory Network (NEON), the Ocean Observatories Initiative, the Advanced Technology Solar Telescope, and Advanced LIGO, a second-generation interferometer—and hopes to receive \$182 million more for them in 2014. That

amount, if achieved, would allow NSF to request \$15 million to \$20 million for LSST without raising the overall size of the account. “I’ve been told we are a high priority for both agencies,” Tyson says. “But it’s hard to read the political tea leaves.”

Funding delays have become a way of life for David Schimel, scientific director of NEON. Sequestration represents yet another

threat to NEON, which was first proposed in 2000 but which officially broke ground only 2 months ago. “We’re constantly rearranging our schedule,” says Schimel about the \$434 million project. “It appears to be a full-time occupation.”

Sequestration notwithstanding, Schimel believes NEON is finally on its way. NSF traditionally favors existing projects over new starts when money is tight, he notes, and two aerial missions this month—one surveying a forested area in Colorado recently devastated by fire and the other a long-studied forest in western Massachusetts—demonstrate the scientific value of NEON. “It’s pretty cool to see these systems in action after such a long time planning them,” Schimel says.

Tyson, soon to become chief scientist under a new management structure, hopes to be able to say the same in a few years about LSST.

—JEFFREY MERVIS



A flying start. Aerial surveillance is part of the arsenal of tools for NEON, a fledgling national ecological network.

tists how to extract knowledge from massive data sets, he says, and give the public “a color motion picture” of the cosmos in unprecedented detail.

LSST’s funding profile holds another ray of hope. The project, to be built atop a mountain in northern Chile, doesn’t need to get its nose inside the NSF construction tent until late in FY 2014. The initial investment would be small, he adds, before ramping up to \$75 million or so in 2015 and subsequent years toward a scheduled completion in 2019.

The NSF money would be used to build the facility’s 8-meter mirror, site infrastructure, and data management systems. The Department of Energy has already agreed to fund a \$160 million, car-sized, 3.2-gigapixel digital camera that will make LSST what Tyson calls “the widest, fastest, deepest eye on the universe.”

Tyson hopes each agency will hold up its

SCIENCE AND THE COURTS

In Mock Case, Biological Evidence Reduces Sentences

Jonathan Donahue is a textbook psychopath. In 2008, he beat the manager of a Burger King restaurant, leaving the man with permanent brain damage. When apprehended, he showed no remorse. He'd kept bashing the manager on the back of the head with a pistol, he said, because "that fat son-of-a-bitch wouldn't stop crying." He bragged about the beating to other prisoners and had a crown—a reference to the fast-food restaurant's cartoon king mascot—tattooed on his back.

In a recent Web-based study, 181 state trial judges from across the United States reviewed the details of Donahue's case, which is fictional but loosely based on a real crime. All of the judges read testimony from a psychiatrist who testified that Donahue had been diagnosed as a psychopath. Some of them also read testimony from a neurobiologist, who presented results of a genetic test showing that Donahue possessed a specific genetic variant linked to violent behavior. The neurobiologist also testified that research with other psychopaths has identified abnormalities in brain function that may under-

logical evidence in such a violent case surprised James Tabery, a philosophy professor at the University of Utah in Salt Lake City, who conducted the study with two Utah colleagues: social psychologist Lisa Aspinwall and law professor Teneille Brown. The trio reports its findings on page 846. Biological explanations of behavior are potentially a double-edged sword in the courtroom, Tabery explains: They could be seen as aggravating evidence that a defendant is "hard-wired" for bad behavior and destined to reoffend, or they could be seen as mitigating evidence that a defendant's behavior was outside his control. Tabery says the new findings suggest the sword may cut more sharply for the defense in such cases.

And the cases are not just hypothetical. To the consternation of some of his colleagues, neuroscientist Kent Kiehl of the University of New Mexico, Albuquerque, testified for the defense in the 2009 sentencing phase of a trial for Brian Dugan, who was convicted for the rape and murder of a 10-year-old girl in Illinois (<http://scim.ag/BrianDugan>). Kiehl testified that brain

scans his team performed on Dugan showed abnormalities in brain activity consistent with psychopathy. "Without the brain imaging stuff the jury would have been back in an hour," Dugan's defense attorney, Steve Greenberg, told *Science* at the time. As it was, they deliberated for 10 hours before returning a death sentence. Neuroscience evidence may have spared another convicted

murderer from the death penalty in a 2010 case (<http://scim.ag/GradyNelson>).

It's very difficult to track how often biological explanations of behavior are introduced in court and how judges and juries weigh them against other types of evidence, says Owen Jones, a law professor at Vanderbilt University in Nashville and director of the MacArthur Foundation Research Network on Law and Neuroscience. "What's

pathbreaking about this paper is that it isolates, as well as one can, both the effects of biological testimony on outcomes and it also does this within a sample of the real-world decision-makers, the judges themselves."

Psychopathy has long been viewed as an aggravating factor in criminal cases, partly because its characteristic traits—including callousness, arrogance, and lack of remorse—aren't sympathetic, and partly because it's one of the best predictors of recidivism. Some researchers consider it a mental illness even though it's not listed in the primary diagnostic manual, and work by Kiehl and others has linked psychopathy to abnormalities in brain regions involved in regulating emotion and behavior (*Science*, 5 September 2008, p. 1284). "Understanding psychopathy as a mental-health disorder changes people's ideas about sentencing," Kiehl says. Developing effective treatments would change them even more, he adds.

But there's a danger in applying published studies showing that the brains of psychopaths differ *on average* from the brains of nonpsychopaths to individual cases, says James Blair, a cognitive neuroscientist who studies psychopathy at the National Institute of Mental Health in Bethesda, Maryland. "I don't think the data are there to do this on an individual basis," he says.

Among the questions the new findings raise for future research is how sentencing might be influenced by biological evidence of other conditions that might be viewed more sympathetically than psychopathy, such as intellectual disability or posttraumatic stress disorder, Brown says. "If you could show some biological risk factor for those disorders, it might reduce sentencing even more," she says.

If judges are, in fact, giving extra weight to biological evidence in real-life cases, the next obvious question is whether they should, says Stephen J. Morse, a legal scholar specializing in criminal law and neuroscience at the University of Pennsylvania. "I think the answer there has to be no," Morse says. For example, he says, if the law deems poor impulse control to be relevant in sentencing, "why should it matter whether that poor impulse control is a product of a biomechanical cause, a psychological cause, a sociological cause, an astrological cause, or any other cause for which the defendant is not responsible?"

—GREG MILLER



Biology goes to court. A new study looks at whether judges are swayed by testimony about the biological basis of criminal behavior.

mine the normal human aversion to inflicting harm on others. When asked to imagine how they would rule in a sentencing hearing, the judges who read this biological explanation cited more mitigating factors (such as mental illness) and handed out modestly reduced prison sentences—13 years, on average, compared with 14 years for judges who did not see this testimony.

That judges would be swayed by bio-

INDIA

Negative Report on GM Crops Shakes Government's Food Agenda

NEW DELHI—Sounding what some regard as the death knell for the development of genetically modified food crops in India, a high-profile parliamentary panel here last week recommended that GM crop “field trials under any garb should be discontinued forthwith,” and that agricultural GM research should “only be done under strict containment.” In a press conference after the report’s release, the panel’s chair, Basudeb Acharya, was unequivocal: “India should not go in for GM food crops.”

If implemented, the report’s recommendations would paralyze research and erode India’s food security, warns India’s chief of crop research, Swapan Dutta, a rice geneticist and deputy director general here at the Indian Council of Agricultural Research. “It would be better if India should end all research on GM crops if the country can’t embrace it,” he says. The government must take a stand on “whether it seeks to embrace or shun biotechnology,” adds vaccine specialist Maharaj Kishan Bhan, secretary of the Department of Biotechnology here. If it comes down in favor of a ban, he says, hope for GM research in India is lost.

Decisiveness won’t be easy, considering that the federal government has been sending mixed signals about its commitment to agricultural GM technology. In 2002, the government gave a green light to the first commercial GM crop in India: cotton carrying the gene for the *Bacillus thuringiensis* (Bt) toxin, which is toxic to some insects. Today more than 1100 Bt varieties account for 93% of all cotton sown in India; production has skyrocketed from 0.02 million hectares in 2002 to 9.33 million hectares in 2011. In February, Prime Minister Manmohan Singh reiterated his support of GM crops in an interview with *Science* (24 February, p. 907). “In due course of time,” he said, “we must make use of genetic engineering technologies to increase the productivity of our agriculture.”

But some of Singh’s own ministers

haven’t been toeing that line. In 2010, former environment minister Jairam Ramesh put an indefinite moratorium on commercialization of Bt brinjal, a kind of eggplant, after the ministry’s scientific advisory panel had given the GM variety a thumbs-up. Then in June, environment minister Jayanthi Natarajan told *Science* that “genetically modified foods have no place in ensuring India’s food security.”

The panel came down squarely on the side of GM skeptics. Chaired by Acharya,



Forbidden fruit. Leaders seek to ban future field tests of genetically modified plants, such as Bt brinjal (inset); Bt cotton is already in use.

a member of parliament representing the Communist Party of India (Marxist), the 31-member panel labored for 2 years on its 492-page report. It blasted GM crops in part on economic grounds, observing that “the experience of the last decade has conclusively shown that while it has extensively benefited the industry, as far as the lot of poor farmers is concerned, even trickle down is not visible.”

GM crop researchers in India were under considerable duress well before the report came out. Since 2011, state governments have refused to issue certificates that would allow GM crop field trials to commence. As a result of this de facto ban, “virtually no new proposals come to us to fund research

on GM crops,” says Bhan, whose department has funded work on 30 kinds of GM crops, from rice to rubber. “Today the pipeline has almost dried up,” he says.

The Acharya panel assailed India’s notable GM success, Bt cotton. It pointed out that all Bt cotton grown commercially in India is derived from technology sold by the multinational food giant Monsanto and incorporated in Indian seed varieties. “It is the fear of multinational control of food security that usually leads to a negative approach on recombinant DNA technology,” says agriculture scientist M. S. Swaminathan, chair of the M. S. Swaminathan Research Foundation in Chennai.

The panel notes that 70% of India’s 1.2 billion people are farmers, who mostly have “no alternative but to buy Bt cotton seed” because the yields are higher. In the last few years,

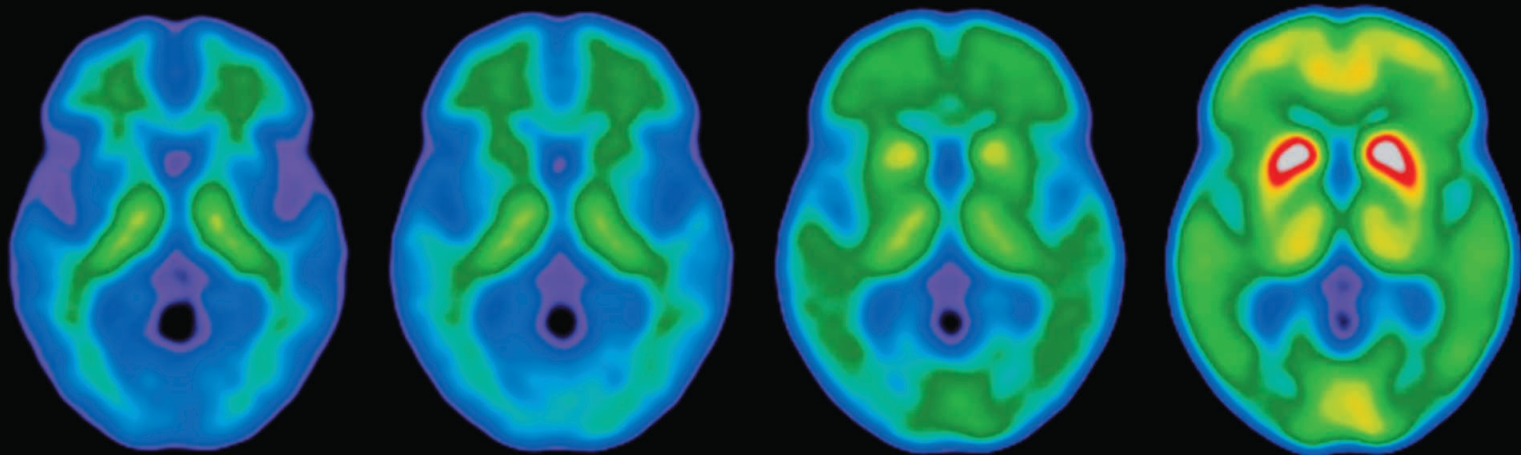
thousands of heavily indebted farmers in India’s cotton-growing regions have committed suicide. In one of its more contentious statements, the panel asserted that “there is a connection between Bt cotton and farmers’ suicides.”

In a statement to *Science*, Monsanto noted that India has reaped big benefits from Bt cotton: “India has seen a cotton revolution with farmers doubling cotton production using better seeds and technologies along with improved farming practices and other agri inputs.” The company did not address the issue of farmer suicide. Taking more direct aim at

the panel, N. Seetharama, executive director of the Association of Biotechnology Led Enterprises-Agricultural Group in Bangalore, said that “the partial and one-sided arguments put forth in the public domain could harm the national interest.”

Ministries now must digest the report and later explain to the panel whether and how they plan to implement the report’s recommendations, which carry political weight but are not mandatory. If the government doesn’t make a forceful case for GM crops, Bhan says, there may be no alternative but to “stop all use of GM crop technology till it has been totally made in India.” And if Monsanto becomes “a nuisance,” he added, “it can be kicked out.”

—PALLAVA BAGLA



Stopping Alzheimer's Before It Starts

Three new clinical trials expected to begin next year will attempt to prevent dementia by treating people at risk for the disease before they develop symptoms

ALZHEIMER'S DISEASE HAS STALKED

Matthew Reiswig's family for generations. His grandfather developed dementia by age 42. "My grandfather had 13 brothers and sisters, and of the 14 kids, 10 developed early-onset Alzheimer's," says Reiswig, a creative director at an interactive firm in Tulsa, Oklahoma. "It's been devastating to my family." Reiswig's father and uncle had symptoms of dementia by age 50, and at age 38, he knows he has a 50–50 chance of developing the disease, probably in the next decade. Those are the odds that he has the gene mutation that runs in his family.

For most people, Alzheimer's disease is a cloud on the distant horizon, a storm that may or may not materialize in old age. But roughly 500 families worldwide live with a more immediate threat: an inherited form of the disease that strikes in the prime of life. Each of these families, like Reiswig's, has a unique glitch in one of three genes. These mutations are cruelly deterministic; those who inherit them are assured of their fate. These families are now the focus of a crucial new stage of Alzheimer's research: They will be subjects in the first clinical trials aimed at preventing the disease by treating people who show no outward signs of sickness.

These trials come on the heels of a decade of bitter disappointment for Alzheimer's researchers, who've seen one promising therapy after another fail in late-stage clinical trials. The latest blow came just last week when Pfizer and its partners announced that they were suspending development of the once-promising treatment bapineuzumab after two large trials found no bene-

fits to mental function in people with mild to moderate Alzheimer's disease. Such failures have raised doubts about the field's guiding hypothesis: that the accumulation of a protein fragment called β amyloid in the brain is a key step in the disease process that ultimately kills neurons and robs people of their memories and the ability to think clearly. Another interpretation, however, is that anti-amyloid therapies have so far disappointed because patients got them too late. If these same therapies could be given years earlier, before irreversible brain damage occurs, perhaps the disease could be prevented.

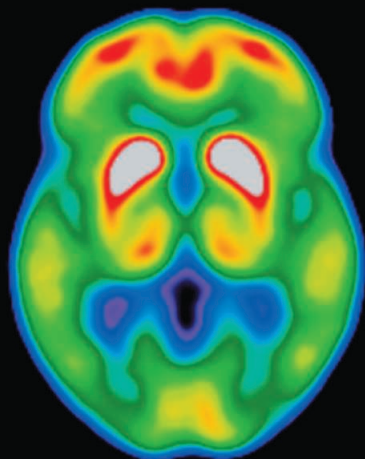
The new trials will put this idea to the test. They will be funded through a combination of support from pharmaceutical companies, the National Institutes of Health, and private philanthropies. Reiswig and his brother plan to participate in a trial affiliated with the Dominantly Inherited Alzheimer Network

(DIAN), a consortium led by researchers at Washington University School of Medicine in St. Louis in Missouri. Another trial, the Alzheimer's Prevention Initiative (API), will focus on an extended family in Colombia. A third trial, dubbed Anti-Amyloid Treatment of Asymptomatic Alzheimer's (A4), will take a different tack, treating adults without gene mutations whose brain scans show signs of amyloid accumulation. All three trials are expected to get under way next year in what should be the sternest test yet for the amyloid hypothesis.



Family portrait. Matthew Reiswig (red shirt, just right of center) and his brother Marty (second adult from the left) plan to enroll in the DIAN clinical trial. Their father, Lawrence (top), has early-onset Alzheimer's disease.

CREDITS (TOP TO BOTTOM): T. BENZINGER AND T. BLAZEWY/WASHINGTON UNIVERSITY; COURTESY OF MATT REISWIG



Gathering storm. Brain scans of people with a gene mutation that causes early-onset Alzheimer's disease, taken at 5-year intervals, show evidence of amyloid accumulation (warmer colors) up to 20 years (far left) before the expected onset of symptoms (far right).

Fighting *la bobera*

Alzheimer's disease affects more than 35 million people worldwide. With that number projected to triple by 2050 as populations age, a preventive treatment would be an enormous boon to public health, not to mention a financial blockbuster. But testing preventive treatments in the general population isn't feasible, for practical and ethical reasons. Because any individual's risk of Alzheimer's disease is relatively low and impossible to predict, such a trial would have to enroll thousands of people and would subject many who would never have developed the disease to the unknown long-term risks of taking anti-amyloid drugs. That's where families like Reiswig's come in: Their deterministic gene mutations make it clear who stands to benefit from an experimental treatment and easier to tell if it's working.

Among the coffee plantations and rural mountain pueblos surrounding Medellín, Colombia, neurologist Francisco Lopera has worked for decades with the largest of these families, which will be the focus of the API trial. Lopera saw his first patient, a 47-year-old man with *la bobera*—the foolishness, as it's called locally—in 1984 as a neurology resident at the University of Antioquia Medical School in Medellín. In the 1980s and 1990s, Lopera traveled extensively through the region, braving drug cartels and guerilla fighters to piece together genealogies. In the mid-'90s, he struck up a collaboration with Kenneth Kosik, a neuroscientist at the University of California, Santa Barbara, that ultimately led to the discovery of the cause of the disease in these families: a mutation in a gene called *PSEN1*. Of the nearly 5000 family members, roughly 1500 carry the mutation.

The API trial in Colombia will test the preventive powers of crenesumab, an anti-

amyloid antibody developed by Genentech, a biotech subsidiary of Roche. One hundred mutation carriers will receive an injection of the antibody every 2 weeks, say API co-directors Eric Reiman and Pierre Tariot of the Banner Alzheimer's Institute in Phoenix. Reiman and Tariot say API chose crenesumab because animal studies suggest it effectively mops up several different forms of β amyloid. Also, crenesumab was designed to avoid an infrequent but potentially worrisome side effect of other anti-amyloid therapies: swelling and micro-

hemorrhages in the brain caused by leaky blood vessels. Genentech's clinical trials in mild to moderate Alzheimer's patients have so far found no evidence of this side effect, suggesting it may be possible to give higher doses safely, Reiman says.

In Colombia, a control group of 100 mutation carriers will receive placebo injections, as will another 100 family members without the mutation. This latter group is necessary because the vast

S Video featuring the progression of amyloid accumulation in the brains of gene mutation carriers at www.scim.ag/amyloid_vid.

tests, but the team will also collect biomarkers, including scans that show amyloid deposition in the brain. The trial is designed to last 5 years. Lopera says the families are eager to participate. After years of studying these families and watching helplessly as people succumb to the disease, Lopera says his team has a new sense of optimism. "For the first time we will be able to offer a therapeutic option," he says.

A multipronged attack

The DIAN trial that the Reiswig family plans to enroll in has a different design. It will include people with mutations in any of the three genes linked to early-onset Alzheimer's: *PSEN1*, *PSEN2*, and *APP*. The first stage of the trial, scheduled to last 2 years, will test three treatments, says the trial's director, Randall Bateman of Washington University. The final decision on what those

compounds will be has not yet been made, Bateman says, but all will target β amyloid, either by slowing its production or clearing it from the brain.

This first stage of the DIAN trial will rely heavily on biomarkers. Recent work by DIAN researchers has begun to provide a picture of the pathological changes in the brain that pre-

Alzheimer's Prevention Trials at a Glance

Trial	Participants	Treatment	Outcome Measures
API: Alzheimer's Prevention Initiative	300 members of Colombian families, including 100 carriers of a mutated <i>PSEN1</i> gene	Crenesumab (Genentech)	Primary: Cognitive. Secondary: Biomarkers, including brain scans to measure amyloid accumulation and brain atrophy
DIAN: Dominantly Inherited Alzheimer Network	240 members of families with early-onset Alzheimer's; 60 have a mutation in one of three genes	Three anti-amyloid therapies to be determined	An initial phase will use biomarkers to identify the most promising drug candidate for a follow-up phase to examine cognitive effects
A4: Anti-Amyloid Treatment of Asymptomatic Alzheimer's	1500 healthy seniors, including 500 with amyloid-positive brain scans	One anti-amyloid therapy to be determined	Primary: Cognitive Secondary: Biomarkers

majority of participants don't want to know whether they carry the mutation, Reiman says. Including the noncarriers makes it possible to blind the trial so that neither the participants nor the doctors treating them will know their genetic status. Mutation carriers as young as age 30 can receive crenesumab if they are within 15 years of their parents' age of onset.

The primary measure of the drug's effect will be changes on a battery of cognitive

cede cognitive problems in people with inherited early-onset Alzheimer's. A study of 128 DIAN participants, reported on 12 July in *The New England Journal of Medicine*, found that concentrations of β amyloid in cerebrospinal fluid dip up to 25 years before the onset of Alzheimer's symptoms. This dip is thought to indicate that the amyloid is being taken out of circulation as it begins to build up in the brain. Brain scans reveal amyloid accumulation and atrophy at least 15 years prior to

onset of symptoms. During the first stage of the clinical trial, researchers will monitor these and other biomarkers, as well as the participants' cognitive performance, and then choose the drug—or drugs—that look most promising for extended testing.

Like API, DIAN will also enroll family members without gene mutations so that participants can remain ignorant of their genetic status. But both trials will pay for genetic testing and counseling for participants who want it. Reiswig is one of the few who's decided to take them up on it. He says he's long assumed he has the mutation and he and his wife have lived accordingly, taking vacations and saving little for retirement. If he tests positive, he says the main thing he'll do is have the kind of heartfelt talks with his children that people often put off. He'll make a video, too, so they'll have a reminder of what their father

was like with a healthy brain and clear mind. They never got that chance with their grandfather, Reiswig says, nor he with his.

Scanning ahead

Unlike API and DIAN, the A4 trial will attempt to prevent the far more common form of Alzheimer's disease that's not caused by



All in the families. Neurologist Francisco Lopera (left) and his collaborator, neuroscientist Kenneth Kosik, pore over genealogies in Colombia.

a gene mutation. The trial will enroll people age 70 or older who test positive on a scan of amyloid accumulation in the brain, explains Reisa Sperling of Harvard University, one of the principal investigators.

A4 will enroll 500 amyloid-positive participants and 500 amyloid-negative controls in a 3-year double-blind trial that will track changes in cognition. Another 500 people with amyloid-negative brain scans will participate in a parallel "natural history" study of aging and cognition. Sperling says the

group plans to select a drug by December, by which time more details should be available from the bapineuzumab trials as well as another closely watched phase III clinical trial for solanezumab, an anti-amyloid antibody developed by Eli Lilly. Even if both drugs fail in people who've already been diagnosed with Alzheimer's, they might still work prophylactically—or at least that's the hope.

A4 will also include an ethics arm that will examine the psychological impact of disclosing information to individuals about their risk of developing Alzheimer's disease (see sidebar). "This is a really important opportunity to study what people hear when they get this information," Sperling says.

High stakes

A great deal hinges on the outcome of these trials. If they fail, it would be a major blow to the near-term prospects of a disease-altering treatment for Alzheimer's disease and perhaps even spell the beginning of the end for the amyloid hypothesis.

Almost any degree of success, on the other hand, would be a major victory. Although the extent to which the early- and late-onset forms of Alzheimer's involve the same mechanisms is still somewhat controversial, many researchers think there are enough similarities that any therapy that prevents or mitigates the early-onset form would at least be a strong candidate for trials in a broader population. A logical step in that direction would be a trial in people with *ApoE4*, a generic variant that increases the risk of late-onset Alzheimer's disease.

Whichever way these pioneering trials turn out, millions of people who aspire to a sound mind in old age have a stake.

—GREG MILLER

How to Talk About Alzheimer's Risk

If Alzheimer's disease were written in your genes, would you want to know? Most people do not, say researchers leading two upcoming clinical trials that will try to prevent Alzheimer's in people with gene mutations that cause an early-onset form of the disease (see main text, p. 790). To accommodate the participants' wishes, those trials will not reveal genetic testing results. But what about less certain risks?

Many people do want to know if they have the so-called *ApoE4* genetic variant, which triples the risk of developing Alzheimer's disease in old age, says Robert Green, a medical geneticist at Harvard University and principal investigator of the Risk Evaluation and Education for Alzheimer's Disease study. Over the past decade, Green and his colleagues have looked at the impact of telling people their *ApoE* status. When people who are psychologically healthy find out they're *ApoE4*-positive, they handle the information fairly well, Green says. "Whether they can do something medically about it isn't as important as you might think," he adds. People who find out they are positive often take other measures, such as buying long-term care insurance or joining an advocacy group, Green says.

A third upcoming trial, the Anti-Amyloid Treatment of Asymptomatic Alzheimer's (A4), will enroll people with a different risk factor: evidence of β amyloid accumulation in the brain, widely thought to be an indicator of the disease. In contrast with genetic testing, however, the predictive value of a β amyloid-positive brain scan is not well understood, says Jason Karlawish, a professor of ethics and health policy at the University of Pennsylvania, who will lead an ethics arm of the A4 trial. To enroll in the trial, participants must agree to find out their amyloid status, and Karlawish says he and colleagues are working on how to explain the scans and convey the uncertainty. Researchers will monitor participants for mood and lifestyle changes and examine how β amyloid test results affect their perceptions of their cognitive abilities and future risks.

With the approval earlier this year of florbetapir, a radioactive compound that binds to β amyloid in the brain and makes it visible on a positron emission tomography scan, more and more doctors will start ordering these tests, Karlawish says. He hopes his study will provide guidance for doctors on how to deliver the news, and on how patients are likely to take it.

—G.M.



On the hunt. GlacierWorks climbers search for the exact locations where early British explorers snapped the original iconic images of mountains and glaciers in the Himalaya.

tell the story the way reams of scientific papers cannot.

In 2007, Breashears formed GlacierWorks, a company that's creating an interactive Web site to allow viewers to navigate Himalayan landscapes constructed from terabytes of high-resolution images. Breashears and his GlacierWorks colleagues are now working with computer scientists and image experts at Microsoft Research (MSR) in

Richland, Washington, and Bangalore, India, to augment the images with numerous other layers of scientific data and models—just as Google Earth highlights roads, borders, and other features atop a seamless tapestry of satellite images. But instead of seeing what Breashears calls “impersonal” flat, two-dimensional satellite images, viewers will be able to peruse vistas from photos taken at eye level, giving them a far better sense of what it's like to travel the Himalaya in person. Along the way, viewers will be able to click on icons superimposed over the landscapes to learn about hydrology and how glaciers accumulate, lose ice, and flow down mountainsides. They'll also be able to work with climate models, changing variables such as CO₂ emissions, population, and the balance of electricity created from coal and renewables to see the effects on glacial melting and river flows in decades to come.

Jeff Dozier, a hydrologist at the University of California, Santa Barbara, and a veteran of a few battles of his own over climate change, calls the approach a “fantastic” way for viewers to uncover the relationships between atmospheric chemistry, snow accumulation, temperature, ice melting rates at different elevations, and the degree to which snow reflects light back into space. “If you help people figure it out for themselves, it often has a more lasting impact,” Dozier says. “We can really use something like this.” Besides, he adds, “the images are just spectacular.”

CLIMATOLOGY

Mountains of Data

A famous mountaineer and Microsoft researchers are blending images, data, and computer models to document how changing climate is affecting the Himalaya

David Breashears has experienced triumphs and tragedy among the mountain peaks at the top of the world. Since 1978, Breashears, 56, has taken part in 45 expeditions to the Himalaya Mountains in southern Asia. He was the first American to climb Mount Everest twice, a peak he has now ascended five times. A filmmaker and photographer, Breashears has also created more than 40 films and won four Emmys. He is perhaps most famous for his 1996 IMAX movie *Everest*; during shooting, five members of three other climbing teams died when a fierce storm slammed into the mountain while they were near the peak.

In his images, Breashears prides himself on portraying the exquisite beauty and drama of his landscapes. But for a man who sees every detail, Breashears readily admits that for the longest time he was missing the big picture. After dozens of his Himalayan journeys, Breashears began to notice a transformation. Large numbers of the more than 35,000 glaciers that interleave this 3000-kilometer-long mountain range were in rapid retreat. “I saw tremendous changes happening, and I was shocked how bereft I was of knowledge,” he says.

After presenting his images to researchers, Breashears found himself swept up in the conflict over whether the changes were due to natural climate variability or climate change. “We didn't know we would be squeezed from both sides the way we have,” Breashears says. “It's taken a toll on us.”

Although reluctant to enter the fray, Breashears says he felt compelled to help others understand the changes he was witnessing. So he decided to do what he does best: combine his mountaineering, photography, and storytelling skills to let his images



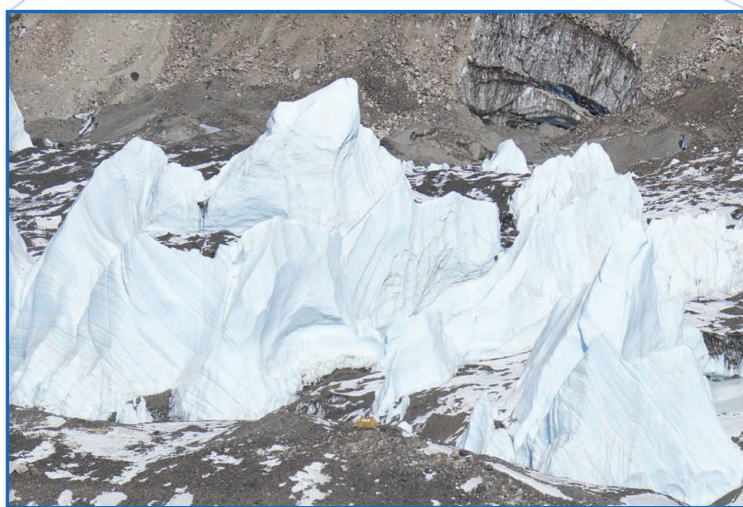
The find. Breashears (right), shown with Mingmar Dorji Sherpa, says it's a “tremendous thrill” to recreate early photos after climbs that sometimes last 20 hours a day.

CREDITS (TOP TO BOTTOM): GLACIERWORKS, 2009; GLACIERWORKS, 2007



Images are indeed the heart of the project: thousands of high-resolution photographs that Breashears and his small climbing team have made during 11 expeditions since 2007. Perhaps the most powerful are “matched” images based on hundreds of original photos taken by British expeditions from the late 19th century through the 1950s. Breashears and his team triangulated the position from which each of the early photos was taken; then they returned to capture an updated view, ideally at the same time of year. In many instances, the glaciers had receded dramatically. “The images have a certain truth to them and a certain power,” Breashears says. “They are irrefutable.”

In another effort, Breashears and his team



went airborne. They mounted seven still cameras aboard a mountain-rescue helicopter and collected 80,000 images in the area surrounding the base camps and ascent routes on Mount Everest, flying as close as possible to

the ground to give the sense of being within the folds of the mountain. Now, working with image experts at MSR and other colleagues, they are stitching together 3500 of the images into a single file that will enable viewers to “fly” up the flanks of Everest near the highest point on Earth.

Still, Breashears wanted to go beyond the images, to incorporate data such as video logs of climbers, displays of scientific studies, and computer models of future melting. No Web program could seamlessly weld such disparate

data sources. In March 2011, however, Breashears spoke with Curtis Wong, an old colleague at the Public Broadcasting Service’s shows *Nova* and *Frontline*, who had since moved to MSR. Wong told Breashears

CREDITS (TOP TO BOTTOM): MAJOR E. O. WHEELER/ROYAL GEOGRAPHICAL SOCIETY, 1921; DAVID BREASHEARS/GLACIERWORKS, 2011 (2)

Downloaded from www.sciencemag.org on August 16, 2012



that MSR colleagues in India were putting together a new Web-based platform called Rich Interactive Narratives (RIN), designed to let users effortlessly move among myriad data types—a wholly new interactive computer experience.

Wong put Breashears in touch with Padmanabhan Anandan, a computer vision expert who runs the Indian lab, and Joseph Joy, RIN's chief architect. By December 2011, Breashears and the MSR team had joined forces to make GlacierWorks fly. "I wanted to work with someone who would push us," Joy says.

Anandan explains that the idea of constructing a player to move between different data types isn't new. It's partly what's behind Google Earth as well as Microsoft's own WorldWide Telescope. But these are closed, one-off systems, useful only within their particular applications. Joy's idea was to create a novel computer language that allowed anyone to integrate all the different data

formats—and thus weave a narrative out of any diverse assemblies of data. In addition to working with GlacierWorks, MSR researchers have teamed up with the Indian government's Science and Technology Department to use RIN to create an interactive narrative of the country's cultural history. And Joy says academics are eager to use the tool to explain science by allowing users to unite numerous studies and data types.

For his part, Dozier says he's excited about the prospect of using GlacierWorks's interactive site to teach students about the uncertainties of climate change. "Glaciers are integrators of climate," Dozier says. The positive side of that is that changes to the glaciers reveal that broad changes in climate are occurring. But that doesn't tell you whether their retreat is due to declining snowfall, faster melting, a combination of the two, or some other factor. So any chance to help students work through those combinations would be a welcome change, Dozier says.

Rivers of ice and time. Panoramic views of the East Rongbuk Glacier on the north side of Mount Everest taken by Major E. O. Wheeler in 1921 and David Breashears in 2011, with a close-up (*left*). Over 90 years, the glacier's vertical height has dropped by 100 meters.

Beyond the educational aspect of the work, Breashears says he hopes the terabytes of high-resolution images he and his colleagues have taken over the past few years will give future scientists a baseline for tracking changes in the size of glaciers, snow accumulation, and melt rates throughout the Himalaya. That may not satisfy the fire-breathing warriors in the political battle over climate change. But that's just fine with Breashears, who says he wants to stand in "the irrational middle" and show people the stark beauty of the highest reaches of our planet and the stark reality of how it's changing.

—ROBERT F. SERVICE



BIODIVERSITY

As Isolation Ends, Myanmar Faces New Ecological Risks

Concerned about the path of foreign investment, homegrown environmentalists seek to protect threatened forests and wildlife and push for sustainable development

GWA, MYANMAR—Bouncing over stone-filled potholes in a four-wheel drive SUV, Myint Aung passes a cluster of huts in a glade hacked from what was once a vibrant bamboo forest. Children in threadbare clothes wave hello. “All these settlements are illegal,” says Myint Aung, a former forestry official who runs Friends of Wildlife, a homegrown nongovernmental organization (NGO) in the capital, Yangon. Here in Taung-nyo Public Protected Forest in western Myanmar, villagers subsist on hunting, illegal logging, and slash-and-burn farming that depletes topsoil. Hectare by hectare, Myint Aung says, they are laying waste to the landscape, where scrub thickets and charred hills stretch for kilometers into the distance.

Until recently, Myint Aung and colleagues could do little more than bear witness to an unfolding ecological crisis. For decades, Myanmar’s military rulers took a dim view of NGOs and outlawed them. But as part of the reforms sweeping the country, the government has cleared the way for NGOs to register legally. Friends of Wildlife is now openly courting foreign donors to support projects aimed at safeguarding biodiversity in Taung-nyo and elsewhere.

Myanmar’s environment is at a cross-

roads, its fate hinging on how recent reforms reshape the country. In his 17 months in office, President Thein Sein has legalized labor unions, rolled back censorship, and released hundreds of imprisoned dissidents. In April, Nobel Peace laureate Aung San Suu Kyi was allowed to run for parliament, winning handily after spending much of the last 20 years under house arrest. Encouraged by those developments, the United States and other countries have eased sanctions on the long-isolated country, opening the floodgates to foreign investment.

Some observers fear that will be bad news for biodiversity: The government, they contend, won’t try to keep developers on a short leash. Others are optimistic that Myanmar’s leaders will embrace a sustainable path. “Before the opening, the only direction for conservation in Myanmar was downwards. We didn’t have the money to defend newly protected areas, and we had no ability to bring international pressure to bear on the government’s environmental policy,” says biologist Alan Rabinowitz, who runs Panthera, a big cat conservancy in New York City. In 2001, he founded Hukawng Valley Wildlife Sanctuary in northern Myanmar. “I see this as a huge opportunity to stabilize Myanmar’s ecology.”

Life lessons. Local environmental advocates have teamed up with international groups to teach villagers how to succeed as farmers.

Running on empty

After 2 decades of international sanctions for human-rights violations, Myanmar lags behind neighboring countries in building an infrastructure. As a result, it retains some of the largest intact forests in Southeast Asia. With 1100 species, the diversity of Myanmar’s birdlife exceeds that of the United States and Canada combined. The country’s Asiatic elephant population—ranging from 1350 to as many as 5300 individuals according to various estimates—is second only to India’s. And Hukawng, a 21,833-square-kilometer swath of jungle in Kachin State, is a haven for many of the country’s 85 remaining tigers. “Myanmar is biogeographically and biologically one of the most exciting places in Asia,” says Peter Leimgruber, a biologist at the Smithsonian Conservation Biology Institute in Front Royal, Virginia.

On the other hand, the sanctions exacerbated the rampant poverty that now makes it harder to protect the country’s ecological resources. From 2001 to 2010, the Wildlife Trade Monitoring Network, or TRAFFIC, in Cambridge, U.K., documented more than 400 tiger, leopard, and Asiatic lion carcasses and body parts for sale in the eastern border towns of Mong La and Talchilek. Tigers face intense poaching threats; a single animal dried and packed in a box can net more than \$30,000 for medicine and other uses in Chinese markets, according to Robert Tizard, a Yangon-based technical adviser to the Wildlife Conservation Society (WCS) in New York City. Leimgruber and Smithsonian colleagues predict that the capture of wild elephants for use as draft animals in the logging trade could extirpate Myanmar’s elephant population within 30 years.

Enforcement is a huge problem. Of the country’s 36 protected areas, just 22 have forestry staff members, Tizard says. “That typically amounts to one or two rangers,” he says. “Not enough to patrol or manage these areas effectively.”

Scant data exist on just what the rangers are supposed to be protecting. Thanks to an agreement with Myanmar’s forestry department, WCS has maintained a low-key presence here since 1993. But neither WCS nor its collaborators have sufficient resources to track environmental changes nationwide. Some areas are too dangerous for fieldwork because of sporadic clashes between the army and rebel groups. A 2012 study funded by the MacArthur Foundation

concluded that Sumatran and Javan rhinoceroses are “probably extinct” in Myanmar—but it couldn’t be sure, because a few rhinos may have found refuge in conflict zones that haven’t been surveyed for years.

Illicit timber trades also cut heavily into the forests. In 2009, Global Witness, a London-based NGO, estimated that more than 90% of Myanmar’s timber exports to China are illegal. China is Myanmar’s biggest trading partner; it imports timber, minerals, and other natural resources, and invests in hydropower schemes. By boosting economic ties with the West, Myanmar may be able to wean itself off that dependency, Rabinowitz says.

Myanmar of late has not shied from sacrificing trade with China to protect the environment. Last October, for instance, the government indefinitely postponed the Myitsone Dam, sponsored by China Power International. Opposed by Kachin rebels, conservationists, and political activists, the \$3.6 billion dam would have flooded 26,000 hectares at the headwaters of the Irrawaddy River, displacing thousands of villagers and degrading habitat of fish and the critically endangered White-bellied Heron. Earlier this year, Thein Sein declared that the dam would never be built during his term in office, which ends in 2015. The government has continued with a string of ecologically favorable policies this year. Last March, it canceled a 400-megawatt, Thai-sponsored coal-fired power plant near Dawei, on the southern coast, on environmental grounds. Then it passed a comprehensive environmental law, the country’s first, which requires environmental impact assessments before the approval of development projects. Mining within 100 meters of Myanmar’s four largest rivers—the Irrawaddy, the Thanlwin, the Chindwin, and the Sittaung—is now banned by a Ministry of Mines decree.

These changes are a positive shift favoring conservation, Tizard says. But it’s unclear how effective some changes will be without substantial investments for conservation and enforcement. “Top-level officials say what they want done, but they don’t supply enough money to carry out their policies,” says one ex-forestry official. Forest rangers on minuscule wages routinely risk their lives confronting well-armed poachers, Rabinowitz adds. WCS is supplying technical and financial assistance to the forestry department to boost wildlife patrols. “What tiger, elephant, and other species in the parks need is protection,” says Than Myint, WCS country program director. “With more patrols, my personal feeling is their numbers will rebound.”

Than Myint and his WCS colleagues have found that illegal hunting earns more than farming for villagers living in or near two protected areas: Hponkanrazi Wildlife Sanctuary on the Indian border, and Hkakaborazi National Park bordering China. Interviews they conducted point to sharp declines in commercially valuable species in these areas, including tiger, otter, musk deer, bear, and pangolin. Most wildlife products leaving Hkakaborazi—including 376 carcasses from 13 species identified during patrols and checkpoints over 8 months in 2006 to 2007 were headed to China, the team reported in *Environmental Management* in 2010 and 2011.

More patrols will curtail the bush meat trade, Than Myint says. But the government, he says, must also confront the root cause

ests in pursuit of Asian bison, or gaur. Using hunting dogs and spears, the warriors would take three or four gaur in a good week. “But they would also take anything else of value they could find,” Myint Aung says. “Cats, tortoises, whatever they could sell.”

Thanks to Friends of Wildlife, the Chin have settled in huts surrounded by cash crops. After an 8-hour drive from Yangon, Myint Aung and two colleagues arrive in Gwa Township to check on the settlers. Myint Aung greets a shy teenage girl who has just completed the equivalent of a high school education. “She’ll be the first Chin family member here to go to university,” he says.

Myint Aung secured 80 hectares of degraded land for the Chin, who have signed onto a stewardship program. In exchange



Getting by. Some Chin families are planting sustainable crops in western Myanmar, where opportunistic hunting was the rule. Charcoal burners (*right*) still practice an illegal trade, burning trees for fuel.



of the wildlife trade: a lack of alternative income sources or land tenure for impoverished villagers. In Myanmar, the state owns all the land, so villagers have little incentive to care for it.

The government has said it intends to change that. In 2001, it pledged to hand 930,776 hectares—about 1.5% of the country’s area—to communities with sustainable management plans by 2031. Degraded land now can be held for 30 years by community groups that agree to restore depleted soil. But villagers lack the resources and organizational capacity to work the land sustainably, says Saw Htun, WCS deputy country program director. For community forestry to succeed, he says, more outside support is needed.

Now that it’s a legal entity, Friends of Wildlife says it can step up efforts to help communities. It’s now working with 24 Chin families whose lifestyle, until recently, was to migrate through Western Myanmar’s for-

for giving up hunting and slash-and-burn farming, the Chin receive schooling and technical support from Friends of Wildlife to help them shift to sustainable agriculture. Camera traps show less human disturbance in the forest now compared to 5 or 6 years ago, says Myint Aung, who hopes that this approach can be replicated elsewhere in Myanmar.

Rabinowitz and others say they’re optimistic that Myanmar’s evolving legal framework can tilt the balance in favor of conservation. Not long ago, the government was among the most secretive in the world. “Now it’s like they’re on C-SPAN,” Tizard says. But Leimgruber cautions that political reforms elsewhere in Southeast Asia paved the way for developers to pounce while NGOs squabbled. “To protect biodiversity in Myanmar,” he says, “everyone’s going to have to work together.”

—CHARLES SCHMIDT

Charles Schmidt is a writer in Portland, Maine.

LETTERS

edited by Jennifer Sills

A Global Perspective on HIV/AIDS

THE SPECIAL SECTION ON HIV/AIDS IN AMERICA (13 July, p. 167) did not explicitly deal with the variations in HIV subtypes and their implications. HIV-1 is phylogenetically divided into three distinct groups—M, N, O (*1*)—and subtype/clade—A to K. HIV-1B, found predominantly in the United States and Europe, is the most studied subtype, even though it represents only about 12% of worldwide infections (*1*). Of the 34 million HIV-1-infected people in the world, about 48% suffer from HIV-1C (2, 3). By discussing HIV/AIDS in America, we indirectly focus on HIV-1B, when we should be making HIV-1C a priority.

HIV-1C has caused millions of deaths. According to the UNAIDS, currently there are about 34 million people living with HIV, including 2.7 million cases of new infection and 1.8 million deaths in 2010 alone. The majority of the cases and deaths were in HIV-1C-prevalent regions (2, 4). However, the true number is likely even higher. Data are underreported because of lack of resources, illiteracy, and ignorance in developing countries, which make statistical and sampling studies of the patients living in core rural areas almost impossible. Associated conditions, such as HIV-associated dementia (HAD), which is apparently more common and severe in HIV-1B patients (5) than those with HIV-1C (6, 7), may also be underreported. The low incidence of HAD in HIV-1C-affected regions such as India, China, and sub-Saharan Africa may be attributable to under-diagnosis due to social stigma, inadequate medical facilities, ignorance, shorter life expectancy, short survival after HIV infection, discordance in clinical prevalence of various opportunistic infections, lack of enough autopsy reports, various host and genetic factors, and abuse of drugs (8, 9).

Our understanding and treatment of HIV-1B may not apply to HIV-1C. There are substantial differences in HIV-1B and HIV-1C viruses, including variation in sequences, structure, antigenic variance, glycosylation, and other interactions (*10*), which collectively result in different replication kinetics and immune responses (8, *11*). In our experience, successful therapeutic strategies in the United States are more specific for the HIV-1B virus and may not be suitable for HIV-1C patients. The “treatment cascade” (*12*), which quantifies the actual number of patients receiving



AIDS in India. An HIV-positive mother of two sits in the women's AIDS ward of Tambaram Hospital.

ing treatment out of the total number of HIV patients, shows that the treatment is not available to all who need it in HIV-1C-prevalent, low-income countries.

HIV-1C infection is rapidly spreading to Europe and America (*13*). Collaborative international projects and interclade HIV research programs may provide a better vision for clade-specific diversity, opening enhanced avenues for developing therapeutics against HIV/AIDS globally. These programs may help to extend preventive services and treatment to all populations in need.

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Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the past 3 months or matters of general interest. Letters are not acknowledged upon receipt. Whether published in full or in part, Letters are subject to editing for clarity and space. Letters submitted, published, or posted elsewhere, in print or online, will be disqualified. To submit a Letter, go to www.submit2science.org.

HIV/AIDS: The Next Generation

LIKE MANY OTHERS, I WAS SWEEPED UP IN THE excitement about the progress we have made in the fight against HIV/AIDS on display at the recent International AIDS Conference in Washington, DC. However, our progress hasn't been universal. In fact, there are a number of areas in which we may be regressing. One is the course of the epidemic among America's youth. Young people between the ages of 13 and 29 have the steepest rise in new HIV infections, compared to stable incidence in other age groups, and account for 39% of all new infections while comprising only 21% of the U.S. population. (1, 2). The CDC estimates that overall, 20% of HIV-positive Americans don't know they are infected, but when youth are isolated from the equation, a staggering 60% have no idea they carry the HIV virus (3).

Who are these youth? As with much of the domestic epidemic in the last decade, new infections occur disproportionately among youth of color, who represent 80% (2) of new infections. The disease has also hit hardest among young gay and bisexual men (2), which paradoxically results in putting many young women at risk for infection. In my clinic in the Bronx, New York, 50% of the clients who identify as young men who have sex with men report also having sex with women (4).

By some definitions, there is a new generation every 5 years. Considering that the youth sitting in high school today will be out in the world in as little as 3 years, it's clear that those of us who are called to educate and care for youth must remain vigilant. We must invest in a continually updated and vigorous prevention, testing, and treatment program that evolves to engage each generation of youth. We must be guided by science and not politics. If we maintain the status quo, we risk losing the next generation of youth to apathy and losing our gains through a false hope in the scope of our progress.

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Maximizing Endangered Species Research

THE U.S. FISH AND WILDLIFE SERVICE RECENTLY prepared a draft Environmental Impact Statement (EIS) to experimentally study the removal of northern barred owls (*Strix varia varia*) as part of the recovery effort for the threatened northern spotted owl (*Strix occidentalis caurina*) (1). The ethical, economic, and opportunity costs associated with the proposed research raise crucial questions about endangered species research. Previous research on interspecies competition, including spotted owl and barred owl interactions (2), has already clearly shown that spotted owls would benefit from the removal of their competitor. As proposed, the new study will merely confirm those results, while failing to address the fundamental problem: how to implement management at a scale that benefits spotted owls.

It is impossible to design appropriate experiments without first identifying feasible management options. The EIS fails to identify how study results would guide management [(2), pp. 5–6]. Removal of barred owls at a scale that will be effective for long-term conservation is not realistic because of the expected high immigration rates of barred owls into removal areas. Conducting the proposed removal experiment before designating feasible management approaches will waste funds that could be used for implementing adaptive management. This might include removal of barred owls to create short-term refugia. Adaptive management could take advantage of the high variation in niche overlap (3) to promote coexistence. Regardless of the approaches taken, conducting the removal experiments will distract researchers, management agencies, and others from identifying and taking feasible management actions in a timely manner.

Going ahead with the removal experiments would likely result in a loss of public trust. The killing of barred owls for research that is unlikely to be informative, the high financial cost to the public, and the opportunity costs associated with postponing adaptive management have little justification. Public support for wildlife research and the U.S. Fish and Wildlife Service in particular may be severely eroded.

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CORRECTIONS AND CLARIFICATIONS

Books et al.: "An uncommonly open approach" by E. Schlager (13 July, p. 156). In the book information, the title should be *Infrastructure*.

Books et al.: "Similarities despite separation" by D. I. Boomsma (13 July, p. 157). In the text of the review, the title should be *Born Together—Reared Apart*.

Reports: "GSK3-TIP60-ULK1 signaling pathway links growth factor deprivation to autophagy" by S.-Y. Lin *et al.* (27 April, p. 477). The following citation was omitted: C. Charvet *et al.*, *Mol. Cell* 42, 584 (2011). The citation was in the originally submitted version of the manuscript but was deleted during revision. The Charvet paper was the first to show that TIP60 was phosphorylated on Ser⁶⁶ by GSK3.

Reports: "Sexual deprivation increases ethanol intake in *Drosophila*" by G. Shohat-Ophir *et al.* (16 March, p. 1351). An author on the original submission, H. Mohammed, was mistakenly omitted from the list of authors by the coauthors in the published manuscript. He has now been reinstated. The correct author list and affiliations are as follows:

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The HTML version online has been corrected.

PHILOSOPHY OF BIOLOGY

Ending Microbial Myopia

Christian Julian Villabona-Arenas

A self-described “philosophical naturalist,” John Dupré aims “to draw—or at least support—philosophical conclusions from science” and to provide critical perspectives on it. In the 16 essays (published over the past decade) collected in *Processes of Life*, he applies his approach to contemporary biology. The volume stresses three points Dupré sees as crucial to understanding biology and evolution: the developmental systems perspective, epigenetics, and the importance of microbes. In its concluding section, he explores how the biological ideas he has discussed affect our conception of human nature.

Dupré (a philosopher of science at the University of Exeter) begins with a trio of chapters that survey his broader view of science, which he sees as produced by people in interaction with nature. He advocates a methodological pluralism and highlights the pitfalls of a purely reductionist enterprise. Given the variety of scientific activities, he wonders how they could be, in any sense, all about the same thing. Because methodologies have been developed in ways as diverse as the kinds of problems researchers pursue, the author outlines a set of epistemic virtues and advises practitioners to try to meet as many of them as possible.

One fundamental argument Dupré offers against reductionism is that biology works with concepts that depend not only on their constituents but also on the larger systems of which they are part. Debates over the nature of species have generated a substantial fraction of the evolutionary biology literature; less attention has been placed on the definition of genes. In addition to the concerns around such issues, there is the central question of what constitutes the individual organism. (With 90% of the cells within our bodies belonging to microbes, there is much to be said for considering that bacterial community as a single composite entity.) The author reminds us “that theoretical consider-

ations are insufficient to determine unequivocally the boundaries of biological objects. Sometimes, perhaps always, this must be done relative to a purpose.” Altogether, Dupré encourages trying to understand life as a hierarchy of dynamic and constantly changing processes. A living world where none of the entities that constitute an organism are static implies an interactive flux subtly different in every iteration but similar enough to be a distinctive process. Because biological concepts are static

abstractions from life processes and different abstractions provide different perspectives on these processes, we face considerable difficulties in reconciling satisfactory general concepts.

Although their scientific importance remains generally unappreciated, microbes

Dupré and coauthor Maureen A. O’Malley (University of Sydney) present a strong case for ending the myopia that leads us to undervalue microbes.

As Dupré and O’Malley note, paying proper attention to microbes has already yielded observations that may force us to rethink fundamental ideas about evolution. In light of the extent of lateral gene transfer, the “tree of life” seems better considered as a net. Studies of cooperation, development, competition, and communication among unicellular organisms have revealed that they can possess many of the characteristics used to define multicellularity. Given that the entities that form lineages are not always the same as those that form metabolic wholes, collaboration may be the central characteristic of living matter. Traditional organisms cannot be seen as “the” biological individuals on which selection operates.” Abandoning some theoretical commitments will open the way for further understanding nature.

The author’s reasons for recognizing the importance of the environment accentuate the relevance of epigenetics and developmental systems theory, areas that for a long time attracted little interest but have now become

Processes of Life
Essays in the Philosophy
of Biology
by John Dupré
Oxford University Press,
Oxford, 2012. 360 pp. \$55, £30.
ISBN 9780199691982.



Boundary-blurring biofilms. If bacteria in dental plaque (shown here in an SEM) “function as a coordinated, spatially organized and fully metabolically integrated microbial community” (1), Dupré wonders why we would “not consider this community, the organized functional whole, to constitute an organism.”

surpass macrobes (a term Dupré advocates for those organisms that are not bacteria, archaea, or protists) in their contribution to evolutionary history: the first three billion years of life on Earth were overwhelmingly microbial. Even today, they include most living things, exhibit a greater metabolic diversity, and inhabit a wider range of environments (including extremely harsh ones); their collaborative enterprise is so extensive that at the heart of every interface between multicellular eukaryotes and the external environment lies a complex multispecies microbial community. In three essays,

very active. Beyond doubt, Dupré emphasizes, the perpetuation of life from one generation to the next requires much more than simply the passage of DNA. He concludes that genomes do not merely store information. Because of their constant dynamic interaction with other constituents of the cell, their capacities depend not only on their sequence of base pairs. More important, those capacities are determined by the systems of which the DNA molecules are only part.

Turning his attention to humans, Dupré criticizes “Evolutionary Psychology.” He argues that evolution has had ample time

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since the Stone Age to shift our behavior and that “the complexity of the developmental interactions between a wide variety of internal and external factors” (processes such as epigenetics, niche construction, and cultural evolution) means our genes cannot produce a “fixed and constant” human nature. He applies similar reasoning in rejecting claims

of genes for race, highlighting the mistake made in thinking of strings of DNA as having specific functions defined only in terms of phenotypic outcomes. For such reasons, he rejects genetic determinism.

Through their thorough arguments, the essays in *Processes of Life* challenge widely held assumptions about biology and evolu-

tion. Dupré provides a view of life grounded in recent research and current understanding. His perspective also reminds us how much we do not know.

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10.1126/science.1224870

SCIENCE AND CULTURE

#FramingTheArtofScience

Melissa A. C. Ingram

Gerald Weissmann's *Epigenetics in the Age of Twitter* is a compilation of essays that intertwine the profound connections of science and art in the context of our modern era, with its rapid proliferation of mobile apps and instantaneous digital chatter. Weissmann (New York University) uses epigenetics, short-term heritable changes in gene expression that are influenced by our environment, to illustrate the selective pressures in the real social network of Western civilization. An avid reader of *The FASEB Journal* may recognize portions of these chapters, as Weissmann has modified his editorials to create a cohesive and entertaining book. It describes current scientific and cultural trends, using little-known stories of obscure scientists as well as common mysteries (such as the correlation between stress and graying hair and whether early Coca-Cola contained cocaine) to illuminate the ongoing challenges scientists face in dealing with scrutiny and criticism, from colleagues and from our broader society.

The book begins with an introduction to the revolution of digital social media, with the growing presence of wikis and Twitter, the formatting of scientific papers, and how tradition is morphing as journals such as PLoS encourage comments and tweets. In a particularly entertaining section, Weissmann treats us to a fictitious set of reactions, including Rosalind Franklin's “YOU PEEKED,” inserted into Watson and Crick's iconic 1953 publication. Elsewhere, he presents other scientific communication issues, such as the relentless need to publish and the gender gap in authorship and editor positions.

Weissmann also does a fantastic job of highlighting the role of women in both the arts

and sciences. He offers excerpts about many prominent women, such as Hypatia, who in fifth-century Alexandria was the only female member of the academy, taught mathematics and philosophy, and was murdered by the mob for all she represented and political jealousy. Other examples—including Marie Curie, Carol Greider, and Elizabeth Blackburn, who have broken the Nobel Prize glass ceiling and inspired women all over the world—call attention to the accomplishments and struggles of women in the sciences.

Each essay provides its fair share of wit and satire, poignantly illustrating faults or

Epigenetics in the Age of Twitter

Pop Culture and Modern Science

by Gerald Weissmann

Bellevue Literary Press,
New York, 2012. 256 pp.

Paper, \$18.95.

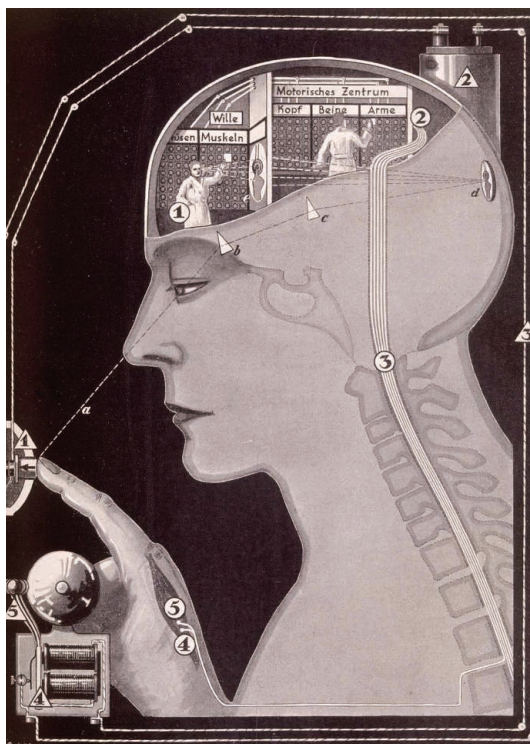
ISBN 9781934137390.

curiosities in current scientific thought or public discourse of the scientific realm. Weissmann, an immunologist obviously well versed in vernacular, even pokes fun at his own field. In the essay “Inflammation Is Complicated: From Metchnikoff to Meryl Streep,” he satirizes the notoriously complex role of inflammation and self-destruction. “Our cells in combat are bathed by evil humors with acronyms worthy of the Pentagon—C5a, C3a, IFN, IL, MIF, etc.—to which they can react in both pro- and anti-inflammatory modes. Signals from ‘helper’ and ‘suppressor’ lymphocytes trigger cellular signals via JAKs and the STATs, the NF-kBs and so forth and so on. The CIA would indeed have trouble deciphering the battle orders.”

Interspersed within are subtle warnings that we ought not be so distracted by the speed of digital chats or the plethora of blogs that we fail to learn from the lessons of the past. Weissmann reminds both current and future scientists how easy it can be to make a difference: “a single observation, by a single scientist, can have a profound impact. It's a gift, so to speak, that's ours for the taking.” The author also cautions readers that while social media and pop culture may influence opinions of science, pop culture is not science—“the bottom line is that the world is round, humans evolved from an extinct species and Elvis is dead.”

This is the age of Twitter, where unrelated ideas, erratic thoughts, and momentary feelings come together to summarize a life story. Or in this collection of Weissmann's essays, how modern science is evolving in the face of ever-increasing social awareness and scrutiny.

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Electronic nerves. From Fritz Kahn's *Das Leben des Menschen* (Kosmos, Stuttgart, 1926).

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CONSERVATION

Citizen Involvement in the U.S. Endangered Species Act

Berry J. Brosi^{1*} and Eric G. N. Biber²

The U.S. Endangered Species Act (ESA) has been controversial since it became law nearly 40 years ago. One of its most-debated provisions is citizen involvement in selecting species that become formally protected under the law (“listing”). Citizens can petition the U.S. Fish and Wildlife Service (FWS) to list any unprotected species and can independently use litigation to challenge any FWS listing decision (1, 2). Some contend that these provisions interfere with the ability of FWS to prioritize scarce resources for species that most need protection (e.g., 3, 4).

Critics charge that most citizen-initiated listings are driven primarily by political motives, particularly to block development projects (5). A related argument is that citizens initiate listing of more subspecies and populations (as opposed to full species) (6), again out of political convenience (5, 7, 8). If such claims are true, citizen involvement may undermine the sole legislative criterion for listing; the ESA stipulates that species should be listed on the basis of biological threat alone, without regard to conflict with development (1). Such criticisms underlie, in part, a 2011 request by FWS to Congress to impose a cap on the amount of money that FWS could spend responding to citizen requests (9).

Although controversy surrounding citizen involvement in ESA listing is longstanding, there has not been an objective analysis comparing species listed by FWS of its own accord to those listed after petition or lawsuit by citizen actors (1). Biological threat provides a test for citizen involvement: If petitioned and litigated species are less biologically threatened, on average, than species selected by FWS, that would provide an argument for reducing citizen involvement in the ESA. Such an argument would be strengthened if citizen groups disproportionately focus on species whose selection might have been based on reasons other than threat, i.e., species that are in conflict with development



The Desert Tortoise (*Gopherus agassizii*). The Mojave Desert population of the Desert Tortoise was petitioned to be listed, but was originally not listed by FWS. The species was listed by FWS after subsequent litigation.

(5), or at “lower” taxonomic levels (subspecies or populations; (10, 11). By contrast, if nongovernmental actors are equally as good as (or better than) FWS at selecting species that are biologically threatened, that would provide an argument for maintaining citizen involvement provisions in the ESA.

Although proposals to constrain citizen petitions in 2001 and 2011 failed in Congress (3, 9), similar proposals are likely to return. To inform this debate, we conducted the first empirical analysis of ESA-listed species that compares FWS-initiated species with species whose listing process was initiated by citizen petition or involved litigation. We asked three sets of questions: (i) Do FWS-initiated species face greater biological threats than citizen-initiated species? (ii) Do citizen-initiated species show signs consistent with what critics deem politically-motivated listing: (a) more conflict with development than FWS-initiated species; and (b) a greater proportion of subspecies or populations as opposed to “full” species compared with FWS-initiated species? (iii) What is the relation between biological threat and both conflict with development and taxonomic status?

Methods

We built a database of domestic terrestrial and freshwater species listed as “threatened” or “endangered” under the ESA (12, 13). Our response variables come from FWS’s recovery priority score, which includes three components: (i) biological threat of extinction; (ii)

Data on listed species refute critiques of citizen involvement in the U.S. Endangered Species Act.

taxonomic level, i.e., full species versus subspecies (including Distinct Population Segments and Evolutionarily Significant Units); and (iii) conflict with economic development (13). We used FWS data from the first recovery report published after each species listing, up until 4 years later; this limits our analysis to species listed from 1986 on. There are 913 species in this data set (14). We only included petitions or litigation whose goal was to list a species (i.e., we did not include lawsuits aimed at delisting species). We validated FWS threat scores with data from a nonprofit conservation organization and used logistic and ordinal logistic regression models. We only included species that were successfully listed under the Act because only these species have recovery priority scores. Exclusion of petitioned species that were never listed under the ESA creates a possible selection bias. To address that possibility, we examined the proportions of petitioned species and FWS candidate species that were actually listed for protection under the ESA (12).

Results

Citizen-initiated species (petitioned and/or litigated) face higher levels of biological threat than species identified by FWS ($P = 0.0005$) (see the figure) (Fig. 1) (table S1). Litigated species are more threatened than nonlitigated species ($P = 0.0027$); we found no significant difference in threat between petitioned and non-petition-initiated species ($P = 0.0930$) (table S1) (15). Citizen-initiated species are more likely to be in conflict with development ($P = 0.0012$) and include a greater proportion of subspecies ($P = 0.0053$) (see the figure) compared with FWS-selected species. This pattern holds in terms of conflict-with-development for petitioned species ($P < 0.0001$) but not for litigated species ($P = 0.1914$). Petitioned species are significantly more likely to be subspecies than non-petition-initiated species ($P = 0.0006$); litigated species are marginally so, compared with nonlitigated species ($P = 0.0567$).

Across all listed taxa (regardless of selection by citizens or FWS), species in conflict with development face greater biological threat levels than species not in conflict with development ($P < 0.0001$) (fig. S1). There is

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no such pattern for taxonomic level: Subspecies and species face relatively similar threats ($P = 0.481$). Of species in conflict with development, citizen-initiated taxa have greater threat levels than FWS-initiated taxa ($P = 0.046$) (see the graph); citizen-initiated subspecies are marginally more threatened than FWS-initiated subspecies ($P = 0.077$) (table S1). Within conflict-with-development and taxonomic-level groups, 11 of 12 comparisons trend toward greater threat for citizen-initiated taxa; 5 show significantly greater threat for citizen-initiated species (table S2).

In terms of the proportion of species that are eventually listed by FWS, we found no evidence of a selection bias in favor of petitioned species compared with non-petition-initiated species. Although we could not conduct statistical tests because of differences in data collection, a higher proportion of petitioned species are eventually listed, compared with species on the FWS candidate list (12). We also found no evidence of systematic divergence between threat scores for FWS and nongovernmental organizations.

Discussion

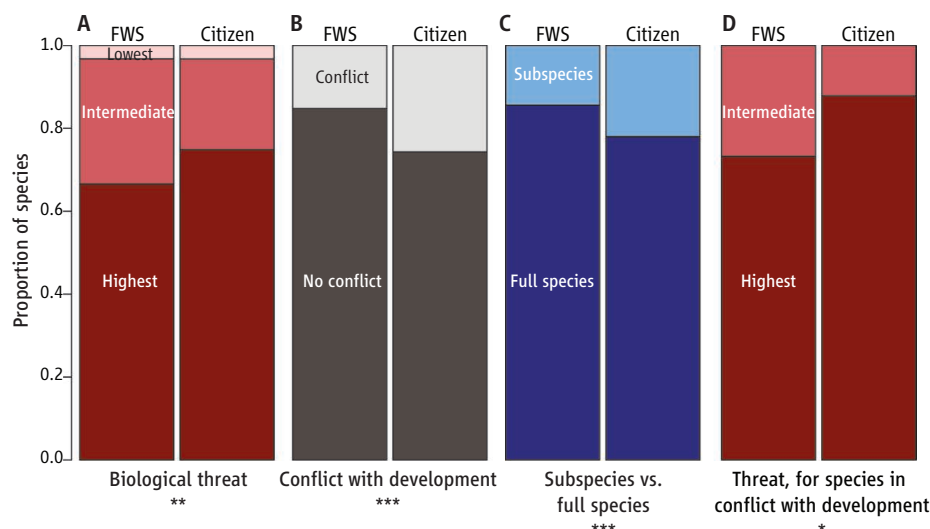
Citizen groups play a valuable role in identifying at-risk species for listing under the ESA. Indeed, citizen-initiated species are overall more biologically threatened than those selected by the FWS. Our findings thus do not support calls for reducing or eliminating citizen involvement in the ESA.

Our results are consistent with potential political motivation (as defined by critics) in species petitions and litigation. First, citizen-initiated species as a whole, and petitioned

species in particular, are more likely to pose conflicts with development relative to FWS-selected species. Second, citizen groups disproportionately propose subspecies, as opposed to full species, for protection under the ESA relative to FWS.

However, even if citizen groups act strategically in their listing proposals, this does not result in listing of species that are less deserving of protection. Petitioned species face levels of threat similar to those of non-petition-initiated species; litigated species face even greater threats than nonlitigated species. Among species in conflict with development, citizen-initiated species are significantly more threatened than FWS-initiated species. Among subspecies, the marginally significant result indicates that citizen-initiated subspecies are at least as threatened as FWS-initiated subspecies, if not more so.

Contrary to criticisms of citizen involvement in the ESA, petitions and litigation are potentially very important in selecting species worthy of protection (16). In many cases, outside groups could serve as the only impetus for protection of biologically threatened taxa that would otherwise be ignored because they conflict with development projects and related political pressures or because they are low-profile subspecies. This function is particularly important because across both FWS- and citizen-initiated taxa, species in conflict with development face significantly greater biological threat levels than species not in conflict with development. This is understandable given that human development projects are one of the largest threats to biodiversity (17).



Comparisons of species selected by FWS versus citizens in terms of (A) biological threat, (B) conflict with development, (C) taxonomic level, and (D) biological threat for species in conflict with development. Results from ordinal logistic (first and last bar pairs) and logistic (second and third bar pairs) regressions; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Citizen actors—including numerous scientists—have specialized knowledge about biological taxa and geographic locales (16). FWS is limited in its budget, staff size, and scope and is unlikely to ever contain enough expertise to identify all species most worthy of protection among the more than 100,000 plant and animal species in the United States, not including subspecies (18). There are structural barriers to listing of taxa that are not truly threatened. Because petitions and lawsuits are time-consuming and expensive relative to the limited resources of many citizen groups, such groups are unlikely to invest time and money in species that probably will not meet the criteria for formal listing by the ESA.

Calls to streamline the ESA and to rely exclusively on FWS to identify and list species might mean that a significant number of species that deserve legal protection—especially those that are politically unpopular because of the potential to obstruct development projects—would be left out in the cold.

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Supplementary Materials

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PHYSIOLOGY

Circadian Time Redoxed

Mino D. C. Belle and Hugh D. Piggins

In all living cells, oxidation and reduction (redox) processes occur as potentially harmful oxidizing or reducing agents (free radicals), such as nicotinamide adenine dinucleotide (NAD⁺) and flavin adenine dinucleotide (FAD), accumulate as by-products of metabolism. In response, biologically active molecules quickly stabilize the relative concentrations of these redox agents. Although redox processes have been associated with cellular house-keeping and homeostasis, recent research has provided a new twist on cellular redox states, linking them to the intrinsic daily (circadian) clock (1–4). On page 839 in this issue, T. A. Wang *et al.* (5) show that the molecular circadian clock and redox states regulate the electrical activity of the neurons that comprise the mammalian central circadian clock.

Circadian rhythms pervade all aspects of our physiology and behavior. For example, at night we sleep and our metabolic activity is low, while during the day, we are awake and active, and our metabolism is high. Genes and proteins that underpin the molecular time-keeper of these rhythms have been modeled as a transcription-translation feedback loop (TTFL). This TTFL clock is present in cells, tissues, and organs of eukaryotes, and some of its molecular components are conserved across animal species. In mammals, the master circadian clock is in the brain's suprachiasmatic nuclei (SCN) of the hypothalamus. Individual neurons of the SCN contain the TTFL clock, and the coordinated activity of these cell-autonomous oscillators conveys timekeeping signals to the rest of the brain and body.

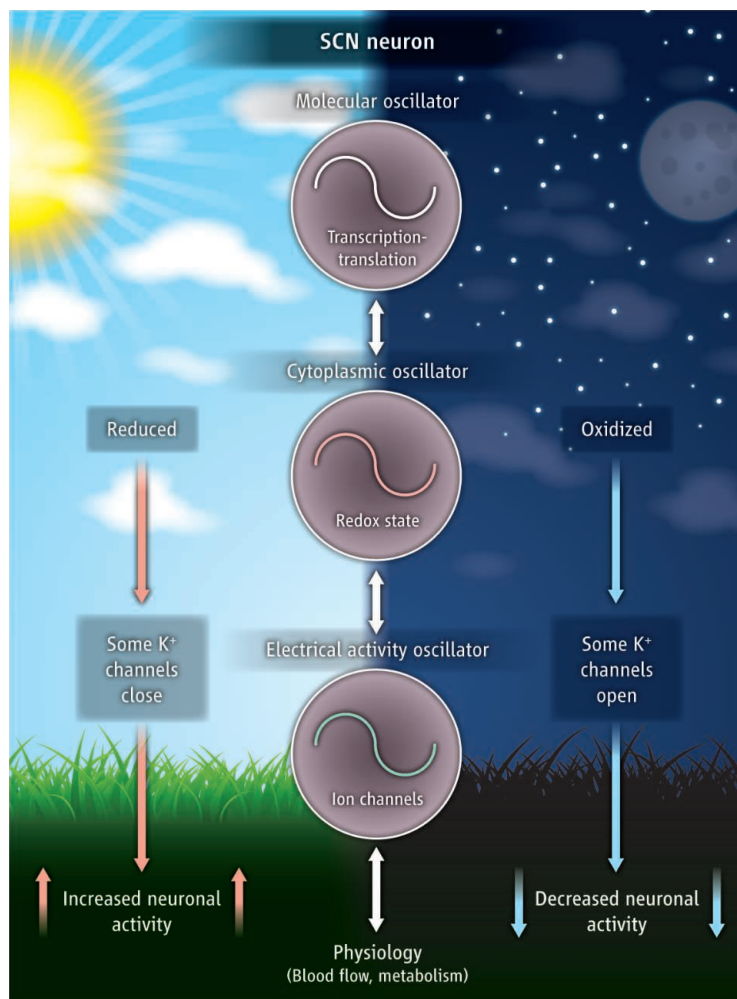
Neurons of the SCN generate daily rhythms in electrical activity, such that the cells are in a more excited state during the day than at night (6, 7). The precise mechanisms by which the TTFL clock drives this electrical rhythm are unknown, but variation in the opening and closing of potassium (K⁺) channels is implicated (8). For example, opening of K⁺ channels at night renders a neuron's resting membrane potential (V_m) more negative, thereby reducing the possibility that it will spontaneously generate and fire action potentials (changes in electrical potential that serves to transmit signals). During the day, some K⁺-channel activity is reduced, thus

Oscillations of the molecular clock and redox states control the electrical activity of mammalian clock neurons.

enabling a neuron to become more active. Remarkably, this rhythm of neuronal excitability is maintained *ex vivo* when the SCN is kept in a brain slice preparation. Although the TTFL clock influences this oscillation (9–11), impairment of the electrical rhythm can feed back and alter the molecular clock (12). So, neuronal membrane activity and excitability are integral to the clock and provide a bidirectional interface between the molecular oscillator and brain signals. Wang *et al.* show that redox state is an important variable in sculpting this 24-hour rhythm in SCN brain cell activity. They demonstrate that this cycling in redox states is driven by the TTFL clock and, startlingly, may form a secondary cytoplasmic oscillator complementary to that of the TTFL clock.

SCN neurons are metabolically more active during the day than at night (13). Using noninvasive methods to assess the relative concentrations of NAD⁺ and FAD in the cytoplasm of rodent SCN neurons, Wang *et al.* established that the redox state in the SCN *in vitro* shows an endogenous circadian rhythm that requires functional molecular clockwork. They further show that this day-night change in redox state directly influences SCN neuronal activity. This provides a decisive pathway through which the TTFL clock influences SCN electrical activity.

Because SCN neurons can sustain their circadian rhythm in neuronal activity and V_m *in vitro*, Wang *et al.* could measure very fast, low-amplitude electrical events that influence the V_m . The circadian oscillation of redox state aligned with the known day-night variation in the V_m of SCN neurons. Further, the night-time oxidized state in the SCN produced conditions that directly influenced the membrane of the cells,



Influential oscillators. The molecular (TTFL) clock and the non-TTFL circadian oscillators in mammalian SCN neurons are illustrated. In this model, the oscillators influence each other during the day and night. Neuron activity feeds back onto the TTFL clock by unknown mechanisms (not shown).

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increasing the activity of specific K⁺ channels, which reduced excitability in the neurons. During the day, the reduced state shuts down some K⁺-channel activity while modulating the conductances of others, thereby increasing excitability in these cells.

Because clock gene transcription is itself sensitive to redox state, the study by Wang *et al.* and recent work in other circadian systems provide evidence for a metabolically sensitive, nontranscriptional pathway to temporally sculpt the clock's transcriptional and electrical machinery (see the figure). However, further work is necessary to establish how, for example, clamping the redox state at an intermediate level affects the molecular clockwork and its eventual electrical output. Also, confirming the observations of Wang *et al.* and others in vivo will be challenging. Because SCN cells do not exist in isolation but are part of a network of tightly packed neurons that can influence the activity of one another, the question of whether metabolic state is the cause or the result of neuronal activity remains unclear.

Nevertheless, the results of Wang *et al.* and the ideas they propose are consistent with several findings that challenge an exclusive TTFL model of the molecular clock.

Recent research has shown that peroxiredoxins, proteins that are present in virtually all living organisms and that function to buffer the intracellular environment, undergo daily rhythms of oxidation-reduction across a range of species, including those whose cells are enucleated (2–4). There are similarities here also to the circadian clock in cyanobacteria, which do not employ the TTFL clock, as well as the clock in plants, which has both TTFL and non-TTFL components (14, 15). Thus, it seems that across many life forms, TTFL and non-TTFL timekeeping processes are not mutually exclusive, and in mammals, they cooperate to orchestrate circadian output of clock neurons.

Although several K⁺ channels in the SCN show circadian variation in the abundance of their transcripts and in their functional activity, the study by Wang *et al.* illustrates how focusing on a direct link between the activity of these channels and the molecular clockwork may have discouraged the consideration of other important possibilities. Indeed, because neurons and subregions of the SCN are heterogeneous, it will be important to determine if redox state differentially influences SCN neu-

ron activity. Similarly, because other brain regions that influence metabolic physiology may also contain a TTFL molecular clock (16), the influence of redox state on cellular activity in these neural structures should be analyzed as well.

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PALEONTOLOGY

Reproduction in Early Amniotes

P. Martin Sander

The conquest of dry land by vertebrate animals began with the evolution of the first four-legged, amphibious animals ~360 million years ago (1, 2). Amniotes originated ~50 million years later (1) and have since become the most diverse clade of land-living vertebrates, including mammals, turtles, lizards, snakes, crocodiles, and birds. Evolutionary changes in reproduction were crucial for the move from the sea via swamps to dry land. However, the reproductive structures and early life stages of amniotes fossilize poorly. Exceptional insights into early amniote reproduction are offered by recent fossil discoveries (3–6). The fact that these fossils come from ancient seas and lakes and not from dry land helps to explain the paradox that there is an older fossil record for live-bearing amniotes than for egg laying in amniotes.

The key evolutionary innovation that enabled amniotes to colonize habitats away from water was the cleidoic egg. Its complex structure added extraembryonic membranes (the chorion and the amnion) and a shell to the primitive vertebrate egg design with its embryo, yolk, and jelly layers (2). These membranes and eggshell enable egg laying and development on dry land. The shell and egg membranes allow gas exchange to and from the developing embryo, letting oxygen in and carbon dioxide out but retaining water. The shell may be either leathery or calcified. Phylogenetic inference shows that leathery shells evolved first; calcified eggshells evolved independently from leathery eggshells at least four times (see the figure).

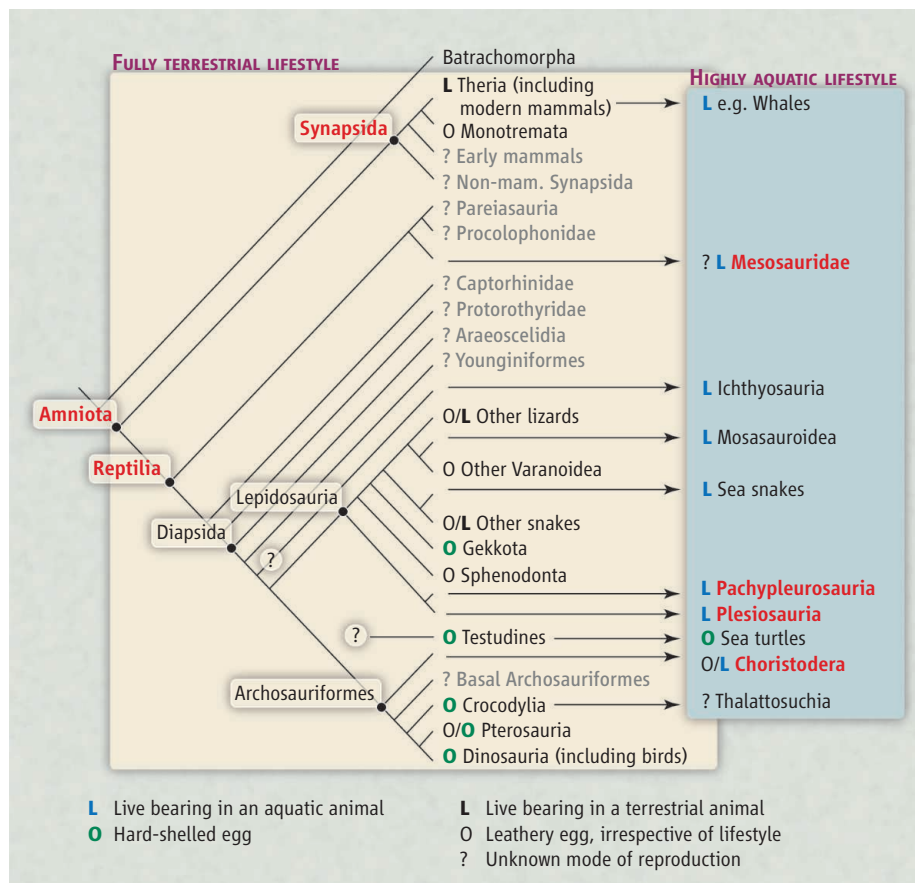
The evolution of the cleidoic egg had two main effects. One was internal fertilization. The other was that eggs could no longer be laid in water, where the embryo would suffocate. This had major implications for the secondarily marine amniotes that frequently evolved from terrestrial lineages.

Recent fossil finds help to explain why the early fossil record is dominated by live-bearing amniotes, although live-bearing amniotes evolved later than egg-laying ones.

Soon after their origin, Amniota split into two major lineages: the mammal-line amniotes (Synapsida) and the bird-line amniotes (Reptilia). Phylogenetic inference from living animals (see the figure) suggests that amniote egg laying evolved no later than in the last common ancestor of mammals and birds, ~310 million years ago (1). Both animal groups have a cleidoic egg, although this has been lost in mammals more derived than monotremes. It is highly unlikely that this complex egg structure evolved more than once (2). Paleontologists have inferred egg laying on dry land from skeletal indicators of full terrestriality—such as well-developed limb joints—in tetrapods close to the mammal-bird split (2).

However, fossils of the cleidoic egg from near the mammal-bird split have been hard to come by. The oldest cleidoic egg fossils postdate amniote origins by 90 million years. At sites in Argentina and South Africa, fossilized egg clutches and embryos of prosauropod dinosaurs have been found that are

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Amniote reproductive evolution and aquatic adaptation. This phylogenetic tree shows the evolution of amniote reproductive traits and the early appearance of live bearing in *Mesosaurus*, as well as the frequent co-occurrence of live bearing and an aquatic lifestyle. Extinct taxa are shown in gray. Extinct terrestrial taxa presumably laid eggs, but fossil data about their reproduction are lacking. Recent evidence of pregnant females and isolated embryos or neonates has been reported for mesosaurs, pachypleurosaur, plesiosaurs, and choristoderes (red) (3, 6). See supplementary materials for the sources used to compile this phylogeny.

220 million years and 188 million years old, respectively (7). The shells of these prosauropod eggs are very thin but sufficiently mineralized to fossilize. This suggests that calcified eggshell had to evolve before the cleidoic egg could fossilize, because a leathery eggshell and the soft contents of an egg do not preserve as a fossil.

In a recent article, Piñeiro *et al.* (6) provide what is likely to be the earliest direct evidence to date for reproduction in amniotes. They report putative pregnant females (see fig. S1) and isolated embryos and neonates of the primitive amniote *Mesosaurus tenuidens* from the Early Permian of South America. Mesosaurs are early reptiles and the first amniotes that invaded the marine habitat by evolving a fully aquatic lifestyle (see the figure) (8). The current fossils (6) come from conservation lagerstätten in Brazil and Uruguay, where finely laminated dark shales and limestone beds were deposited in an epicontinental sea about 280 million years ago (8).

The fossils are adult skeletons with very small and immature skeletons inside the rib cage, as well as coiled-up, small immature skeletons found with adults or in isolation, presumably representing embryos and neonates (6). The fossils are incomplete and partially disarticulated and the sample size is small (6). However, very similar occurrences in geologically much younger amniotes strengthen the case that the *Mesosaurus* specimens are embryos.

The similar but better-preserved and better-studied associations of pregnant females and isolated embryos or neonates are for three species: the 235-million-year-old pachypleurosaur *Neusticosaurus* from the European Alps (9); another pachypleurosaur of a similar age, *Keichousaurus*, from China (3); and the 120-million-year-old choristodere *Hyphalosaurus* (4), also from China. The pachypleurosaur are known from similar laminated sediments as *Mesosaurus*; they resemble it in body plan and were specialized arthropod feed-

ers. The body plan of *Hyphalosaurus* (4) is strikingly similar to those of mesosaurs and pachypleurosaur, but it lived much later.

Neither *Mesosaurus* nor the pachypleurosaur and *Hyphalosaurus* could move on land to any extent. All share the same distinctive adaptation to a fully aquatic lifestyle, namely an increase in bone mass that affords neutral buoyancy by balancing out the tetrapod lung, allowing the animal to move effortlessly in the water column (10).

Another adaptation crucial for a fully aquatic lifestyle was live bearing, because egg laying requires some ability to go on land. Mammals had evolved live bearing long before returning to the sea, but the various clades of marine reptiles evolved live bearing independently and at different times (3, 5, 6, 11). (Sea turtles are the notable exception, illustrating the problems of a marine lifestyle combined with egg laying.) As in modern lizards, live bearing in *Mesosaurus*, pachypleurosaur, and *Hyphalosaurus* must have evolved via embryo retention in the reproductive tract of the mother past the hatching stage; the associations of isolated embryos and adults may reflect incomplete live bearing. Fully developed live bearing is, however, evident in ichthyosaurs (5, 11) and has recently been established for plesiosaurs (5). The large size of the plesiosaur fetus suggests a reproductive strategy as in modern whales for these animals (5).

The relatively rich fossil record of pregnant amniotes (3–7, 11–13), starting with the Middle Triassic ichthyosaurs ~240 million years ago (11), supports the interpretation of the geologically much older *Mesosaurus* finds (6) as embryos inside of females instead of as last meals of cannibals. Cannibalism is well known in modern reptiles, but this is unlikely for the new *Mesosaurus* fossils, for reasons established by the study of other marine reptile finds with small skeletons inside large skeletons of the same species that have been interpreted as pregnant females (3, 5, 11, 12, 13).

Given that live bearing is documented so much earlier and more frequently in the fossil record than egg laying, it might be thought that the primitive mode of amniote reproduction is live bearing and that the amniote egg with its eggshell and extraembryonic membranes evolved by embryo retention (14) and not egg laying. However, several arguments can be made to counter this assumption. First, egg laying is the primitive state for both mammals and reptiles, but primitive eggs with their leathery shells are unlikely to be preserved. Second, observations on lizards show that live bearing evolves readily

from egg laying (by embryo retention) but not the other way around. Finally, a key reason for the rich fossil record of live bearing is the exceptional preservation in conservation lagerstätten, which preferentially sample marine environments and secondarily aquatic amniotes, which were often live-bearing (3–6, 9, 11, 13) (see the figure). Even terrestrial live-bearing mammals are best known from such lagerstätten (12). Further fossils of early amniote embryos and eggs will be invaluable for further elucidating the evolution of this important clade.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/337/6096/806/DC1
Fig. S1
References

10.1126/science.1224301

MOLECULAR BIOLOGY

A Swiss Army Knife of Immunity

Stan J. J. Brouns

Selfish genetic elements are more than a daily nuisance in the life of prokaryotes. Whereas viruses can multiply by reprogramming host cells, or integrate in the host genome as “stowaways,” conjugative plasmids (transferable extrachromosomal DNA) make cells addicted to plasmid-encoded antitoxin factors, thus preventing their disposal. Bacteria and archaea defend themselves against these invasive elements using an adaptive immune system based on clustered regularly interspaced short palindromic repeats (CRISPRs). On page 816 in this issue, Jinek *et al.* (1) show how the CRISPR effector enzyme Cas9 from bacteria is directed not by one, but two small RNAs to cleave invader DNA.

The CRISPR system integrates short DNA fragments from viruses and plasmids into a specific repeat locus of the host cell genome to function as a memory of past invasions. This locus of the “cell’s most wanted” is then transcribed into RNA (the precursor CRISPR RNA), which is cleaved in each repeat to yield individual mature CRISPR RNAs (crRNAs). These guide a dedicated set of CRISPR-associated (Cas) proteins to their targets during cellular surveillance of the cytoplasm for either foreign DNA

or messenger RNA (mRNA) of known invaders. Once identified, foreign nucleic acids are permanently damaged by Cas nucleases, thereby neutralizing the invader (2).

The CRISPR field was set in motion 5 years ago by the discovery that lactic acid bacteria become highly resistant to virus infection when they incorporate virus DNA fragments in their array of memorized invaders (3). Bacterial resistance to the virus is

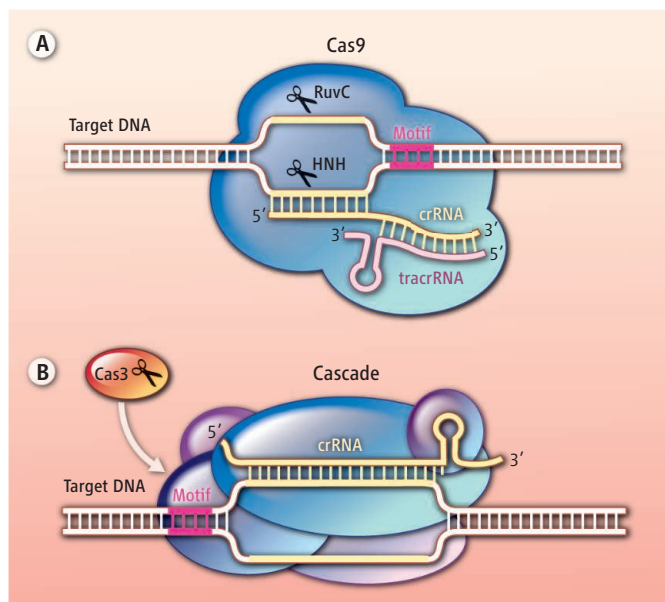
A duplex of two small RNA molecules directs the destruction of intrusive foreign DNA in bacteria.

based on breaks in the viral DNA within this memorized region, and the bacterial gene *cas9* encodes the enzyme responsible (4, 5). However, the modus operandi of Cas9 has remained unknown.

One aspect that had to be resolved first was the unusual way in which Cas9 obtains the mature crRNA. Whereas most CRISPR-Cas systems involve a dedicated nuclease that cleaves the precursor CRISPR RNA in

each repeat (2), Cas9-based systems also require a CRISPR-specific small RNA. This so-called trans-activated crRNA (tracrRNA) base pairs with each repeat of the CRISPR transcript and provides a substrate for the RNA-specific host ribonuclease RNase III (6). The cleavage product, an RNA hybrid consisting of a 42-nucleotide crRNA and a 75-nucleotide tracrRNA, was deemed to be the guide for Cas9.

With this in mind, Jinek *et al.* could show that Cas9 from the human pathogenic bacterium *Streptococcus pyogenes* binds and cleaves invader DNA within the remembered region. Although the site specificity was solely determined by the guiding ability of the crRNA, binding and cleavage of the target DNA surprisingly required the tracrRNA. The tracrRNA thus enables the Cas9-crRNA complex to locate a DNA sequence complementary to the crRNA in the cellular tangle of DNA (see the figure), providing



All-in-one nuclease. (A) Cas9 requires a crRNA and tracrRNA to recognize invader DNA sequences by hybridizing the guide section of the crRNA to one strand of the target DNA to form an R-loop. The flanking motif is critical for this process and may facilitate DNA duplex unwinding and strand invasion by the crRNA. Target DNA is then cleaved by both nuclease domains of Cas9. (B) Cascade-like complexes contain a single crRNA and up to five different Cas proteins. Identified invader DNA sequences are progressively unwound and cleaved by the action of the recruited nuclease and helicase Cas3 (11, 12).

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yet another example of the crucial roles that small RNAs play in cells (7).

Cas9 creates blunt-ended lesions in target DNA by using two nuclease domains, each cleaving one DNA strand of the target double-stranded DNA R-loop. Whereas the HNH-nuclease domain cleaves the DNA strand that base pairs with the crRNA, the RuvC-nuclease domain cleaves the displaced strand of the DNA. Jinek *et al.* show that cleavage was robust and occurred with multiple turnovers in both relaxed and supercoiled DNA targets, implying that Cas9 is functionally recycled after cleavage to destroy more invader DNA copies that may be present in the host cell.

Despite the seeming efficiency of this cleavage and recycling process, the Achilles' heel of Cas9 was also uncovered. Viruses escape immunity by making point mutations in either the memorized regions of their genomes (8), or just outside this region in a conserved nucleotide motif. When testing these mutant DNA molecules, Jinek *et al.* found that binding and cleavage by Cas9 was severely compromised, suggesting that these mutated virus DNA molecules adopt a stealth mode inside the cell and require a new cycle of memory formation before they

are subject to interference once again. Cycles like these contribute to the ongoing coevolution between invaders and their hosts.

Jinek *et al.* realized that a highly specific, customizable RNA-directed DNA nuclease could be useful to edit whole genomes. Based on the 20-nucleotide guide section of the crRNA, the enzyme could theoretically introduce breaks at unique sites in any eukaryotic genome. As a proof of concept, the authors programmed Cas9 to cleave a plasmid carrying the gene encoding green fluorescent protein at predetermined loci using a single chimeric crRNA containing just the critical segment of the tracrRNA. DNA breaks induce cellular DNA repair pathways (9) and this can be harnessed to disrupt, insert, or repair specific genes of cells. Introducing DNA breaks at desired loci using just Cas9 and a chimeric crRNA would be a substantial improvement over existing gene-targeting technologies, such as zinc finger nucleases and transcription activator–like effector nucleases, as these require protein engineering for every new target locus (10). Efficient gene repair strategies in cells from patients, and the reintroduction of repaired cells, could become increasingly important for treating many genetic disorders.

Cas9 is thus a remarkably compact and multifunctional enzyme compared to CRISPR effector complexes from other bacteria or archaea. These are typically 350- to 450-kD crRNA-protein complexes and contain up to 11 protein subunits encoded by four to seven different *cas* genes (see the figure) (2, 11). Yet, nuclease activities are not always part of the complex and need to be recruited when the target DNA is identified (12). With all its activities at hand, Cas9 is truly the Swiss army knife of CRISPR immunity.

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ATMOSPHERIC SCIENCE

Water Vapor in the Lower Stratosphere

A. R. Ravishankara

Water vapor in the stratosphere originates from the troposphere by transport of water vapor itself (1) and of methane and hydrogen, which degrade to make water (2). Water, methane, and hydrogen are transported to the stratosphere through upwelling in tropical regions (3). This upwelling followed by downwelling and horizontal transport in the extratropical stratosphere—the Brewer-Dobson circulation—is widely held to control the water vapor abundance in the stratosphere (see the figure). But could there be a more direct transport of tropospheric air containing water vapor into the stratosphere via convection outside the tropics (4–7)? On page 835 of this issue, Anderson *et al.* (8) argue that there

is evidence for water vapor enhancements in the mid-latitude lower stratosphere. They further argue that increased water vapor levels could enhance ozone depletion caused by human-emitted ozone-depleting substances and thus raise ultraviolet radiation levels at Earth's surface.

Water is a powerful greenhouse gas. It accounts for 50 to 75% of the greenhouse effect today. The amount of water vapor in the tropopause (~10 to ~18 km above Earth's surface depending on latitude) and the lower stratosphere is particularly crucial, because it determines how much radiation escapes from the atmosphere. Clouds also play a role in Earth's radiative balance by decreasing the amount of incoming solar radiation that reaches Earth's surface and by preventing escape of infrared radiation to space. Precipitation redistributes water vapor, and both cloud formation and pre-

Water vapor injection into the stratosphere at mid-latitudes may sporadically enhance local stratospheric water vapor concentrations to high values.

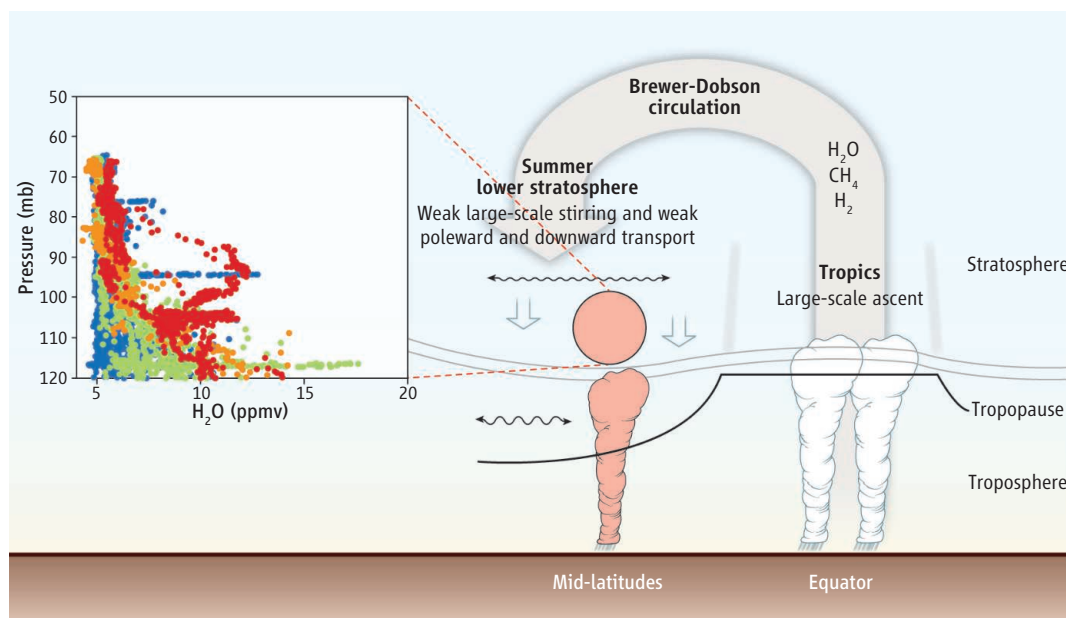
cipitation lead to vertical heat transport in the atmosphere.

Water thus has major consequences for the radiative balance and heat transport in the atmosphere. In addition, it is the source of OH radicals which catalytically destroy ozone in the stratosphere. Furthermore, water droplets and frozen water particles provide surfaces and liquid media for heterogeneous and multiphase reactions (9).

Earth would be a different planet without all these well-known roles of water. Yet, many of these known roles remain unquantified. The potential stratospheric ozone depletion by mid-latitude water injection is a case in point.

The role of water vapor in ozone destruction cycles and hence the maintenance of the ozone layer has been known since 1964, when Hampson suggested (10) that the hydrogen oxides OH and HO₂ drive catalytic

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What determines water vapor levels in the stratosphere? The Brewer-Dobson circulation (light colors) transports to the stratosphere water vapor as well as methane and hydrogen, which produce water vapor [adapted from (3)]. Anderson *et al.* argue for an additional mechanism (red) that directly injects water at the mid-latitudes. Through this mechanism, water vapor in the lower stratosphere could be enhanced sporadically to high concentrations at some locations, until they mix with the rest of the stratosphere. Key questions remain: How pervasive is this mechanism? How will it change in the future? How does it influence the ozone layer recovery? How will it influence climate change and variability? ppmv, parts per million by volume.

ozone destruction cycles. Indeed, the specter of human-induced ozone layer depletion was first raised in connection with the injection of water vapor into the lower stratosphere by supersonic aircraft (11). Anderson *et al.* now argue, based on the observed enhanced water vapor levels, that episodic extratropical convective transport of water can swell and dilute the sulfate aerosols, thereby increasing the rate of heterogeneous activation of chlorine from the benign chlorine reservoirs (ClONO_2 and HCl). These enhanced active chlorine levels can then accelerate ozone destruction. However, the enhanced water abundances are localized in space and time and may not be pervasive. The extent of such events and their contribution to patches of high levels of water are not yet quantified.

The current estimate of the global and latitudinal ozone layer depletion, based on observations, must already include the influence of this mechanism. This influence could be subtle enough to have avoided detection by the observed column ozone levels or surface ultraviolet changes. However, it should be possible to estimate its contribution through retroactive analyses of the observed column ozone and local water vapor values at locations and times where convective transport is likely to have occurred.

How could the mid-latitude mechanism influence the recovery of the ozone layer? The main driver for the expected “recovery”

of the ozone layer—when the ozone layer will return to 1980 levels—is the decline in the atmospheric concentrations of ozone-depleting substances, with secondary influences of climate change (cooling of the stratosphere) and changes in the composition of the troposphere. The mid-latitude mechanism could be another secondary influence on the recovery of the ozone layer, if the frequency and extent of these extratropical injection changes in the future due to climate change. If this process weakens, the recovery should occur earlier than the current estimate; if it gets stronger the recovery could be later than the current estimate.

Elevated water levels increase activation of chlorine and suppress active nitrogen oxides through heterogeneous reaction; they act together to increase ozone destruction at today’s chlorine abundance. The impact of Anderson *et al.*’s mechanism on the recovery of the ozone layer depends on the future extent of extratropical water injection as well as the interplay between these heterogeneous reactions when levels of chlorine (and bromine) are lower. Chemistry-climate model studies that can faithfully capture the extratropical water injection would help bound the contribution of this proposed mechanism and estimate its influence on the recovery of the ozone layer.

The change in the predicted date of global ozone recovery is unlikely to be large because

the extratropical water vapor injection does not appear to contribute greatly to the water abundance in the lower stratosphere on the global scale. Whether this mechanism could alter the influence of large volcanic eruptions or “geo-engineering” injections of sulfate aerosol and the subsequent activation of chlorine and suppression of nitrogen oxides remains to be shown. The mechanism is not expected to influence the Antarctic ozone hole or the large springtime Arctic ozone losses.

There are other important considerations with regard to potential enhancements in lower stratospheric water vapor. For example, the observed trend in the lower stratospheric water vapor contributes significantly to the slower than expected surface temperature rise over the past

decades (12), highlighting the importance of water vapor in determining the extent of heat loss from the planet. Yet, accurate measurements and distributions of the abundances of water vapor in the lower stratosphere and understanding of the mechanism of transport are currently insufficient for accurate accounting and predictions. The suggestions of Anderson *et al.* adds to the list of reasons why we need more accurate measurements and fuller understanding of water vapor transport mechanisms in the lower stratosphere.

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PHYSICS

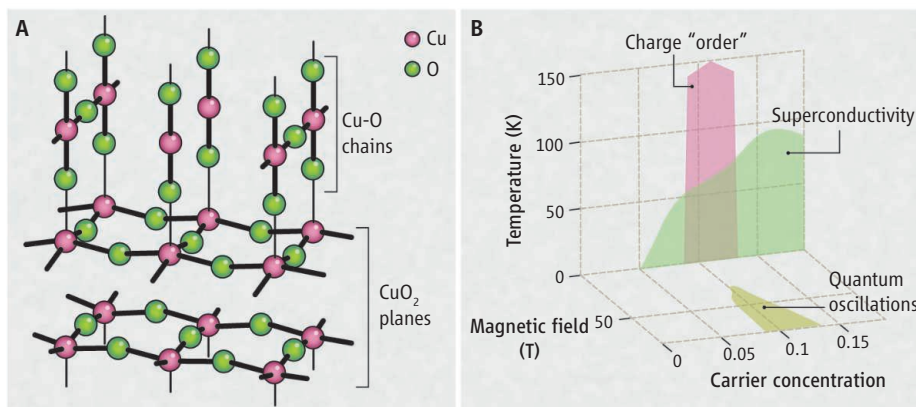
Cuprates Get Orders to Charge

John M. Tranquada

A quarter century after their initial discovery, copper oxide high-temperature superconductors (HTSs) continue to fascinate and frustrate researchers. One of the big challenges to understanding these materials is that the standard model of electron motion in solids—the same model that had to be developed before Bardeen, Cooper, and Schrieffer could formulate their theory of superconductivity in 1957—does not apply, and no convenient replacement is yet in sight. While theoretical efforts proceed, the focus of research has periodically shifted from one experimental surprise to another. For example, several years ago it was discovered in the superconducting compound $\text{YBa}_2\text{Cu}_3\text{O}_{6+x}$ that when the superconducting order is suppressed by a very strong magnetic field, various electronic responses oscillate with the field strength in a way that indicates the presence of a new electronic ordering (1, 2). These experiments stimulated a great deal of theoretical excitement, but a compelling explanation has been lacking. On page 821 of this issue, Ghiringhelli *et al.* (3) report the experimental observation of a specific charge ordering more complicated than generally imagined. Together with an independent study of this order in a magnetic field (4), an explanation for the quantum oscillation experiments appears to be at hand. At the same time, these results yield new puzzles.

Although the driving motivation is to determine the microscopic explanation of high-temperature superconductivity, a big challenge is to understand the high-temperature metallic phase (above the superconducting transition temperature, T_c) from which the superconductivity develops (see the figure). The electrons in a solid interact with each other via Coulomb and exchange interactions, creating a complicated electronic liquid; nevertheless, in the standard model of solid-state theory, Fermi liquid theory, one transforms from electrons to an effective set of independent quasiparticles.

Fermi liquid theory is extremely effective for calculating the properties of many metallic materials; unfortunately, it fails when applied to the cuprates. To understand why,



Superconducting behavior. (A) Arrangement of Cu and O atoms in $\text{YBa}_2\text{Cu}_3\text{O}_{6+x}$. (B) Schematic comparison of the carrier concentration dependence of the observed (fluctuating) charge order (in zero magnetic field) and quantum oscillations (low temperature and high magnetic field).

we turn to the HTS parent materials, such as $\text{YBa}_2\text{Cu}_3\text{O}_6$, which contain CuO_2 planes. Normally, the conduction electrons would lower their energy by spreading throughout the planes, but here the Coulomb repulsion between electrons is strong enough to localize them, making the material an electrical insulator. A consequence of the localization is antiferromagnetism, whereby single electrons in half-filled Cu 3d orbitals on neighboring sites align their spins in opposite directions. In the case of $\text{YBa}_2\text{Cu}_3\text{O}_{6+x}$, the density of charge carriers is adjusted by adding oxygen atoms (by an amount x) to the Cu-O chain layers. Above a threshold level of added oxygen, the material becomes metallic; however, the antiferromagnetic spin correlations survive dynamically. Furthermore, the measured density of charge carriers reflects only those induced by the added oxygen; the initially localized conduction electrons do not appear to delocalize in response to the chemical modification. Although there are many theoretical proposals to describe this unusual “normal” state, no new simplifying paradigm has yet appeared.

The normal state is usually studied at temperatures above the superconducting transition, but one can also cool into the superconducting state and destroy the superconducting order by applying a strong magnetic field. It came as a shock when such measurements on $\text{YBa}_2\text{Cu}_3\text{O}_{6+x}$ first revealed evidence of quantum oscillations in electronic responses, because the observed behavior appears to be well described on the basis of Fermi li-

Modulations in the charge density provide a clue while adding complexity to the story of high-temperature superconductivity.

quid theory. There is a catch, though; application of the standard model to the results implies that the magnetic field causes a radical change in the electronic structure (5).

Many models of the electronic ordering have been proposed, especially models of spin or charge stripe order (6, 7), in which antiferromagnetic correlations and charge density modulations form an interlocked pattern. Similar electronic properties have been demonstrated in $\text{YBa}_2\text{Cu}_3\text{O}_{6+x}$ at high magnetic field and in cuprates that exhibit stripe order at zero magnetic field (8). Recent nuclear magnetic resonance (NMR) experiments indicate the onset of charge ordering in $\text{YBa}_2\text{Cu}_3\text{O}_{6+x}$ in a magnetic field of 15 T (9).

If there is periodic charge order, one would expect to see it by diffraction. Ghiringhelli *et al.* provide the first such evidence. To enhance the sensitivity, they performed soft x-ray scattering at photon energies resonant with Cu 3d levels on a spectrometer capable of resolving inelastic signals. The big surprise is that the modulation is in two directions, not just one as for stripes. At lower temperatures, the charge modulations are correlated over 16 lattice spacings, suggesting that they are almost ordered—but the lack of splitting of the NMR signal in a small magnetic field suggests that the charge modulations may be dynamic. The signal shows up at temperatures at least twice T_c , but only for the limited range of carrier densities at which quantum oscillations have been observed.

Independent work with very hard x-rays, and measuring in magnetic fields of up to 17

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T (4), confirm the two modulation wave vectors, providing further details on the correlations and showing that below T_c , the applied field enhances the intensity of the scattering peaks (with the correlation length reaching 25 lattice spacings). Thus, it appears that the competing order responsible for the quantum oscillations has been detected, and details will likely soon be sorted out.

But this answer to the quantum oscillation problem leads to new questions. Although there has been a proposal regarding fluctuating charge modulations (10), many theorists believe that antiferromagnetic spin fluctuations play an important role in the mechanism of high-temperature superconductivity (11); however, the present charge modulations have no obvious connection with the observed spin modulations, especially the uniaxial spin modulations reported at lower doping (12). Have Ghiringhelli *et al.* detected a type of charge modulation that is universal among cuprates? Or does it depend on unique features of the $\text{YBa}_2\text{Cu}_3\text{O}_{6+x}$ structure? Do the indications of “conventional” electronic behavior revealed by the quantum oscillation experiments point to a hidden simplicity? Or do we still have to struggle with the challenging problem of strongly correlated electron physics? Whatever the answers, the work by Ghiringhelli *et al.* will surely stimulate more investigations in a fascinating field.

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MATERIALS SCIENCE

Order from Disorder

Dongbo Wang¹ and Alejandro Fernandez-Martinez²

Our understanding of the atomic structure of materials relies on our ability to describe structural characteristics such as the short-range order (in the case of liquids or amorphous materials) or the periodicity inherent to crystalline materials. On page 825 of this issue, L. Wang *et al.* (1) challenge our understanding of the inherent disorder that can be present in a crystal by presenting evidence for a crystalline material composed of amorphous clusters. They show that C_{60} molecules from the crystalline solvated fullerene phase C_{60} **m*-xylene undergo an order-to-disorder transition under compression at ≈ 35 GPa but keep their translational symmetry. A material can still possess long-range order even though its fundamental building blocks are disordered.

The resulting phase—a crystal composed of amorphous clusters or ordered amorphous carbon clusters (OACC)—showed both the x-ray diffraction reflections typical of a hexagonal close-packed (hcp) lattice and the broad oscillations of diffuse scattering typical of amorphous materials. Raman spectra indicate a high degree of disorder at the subnanometer scale of an amorphous component, and inelastic x-ray scattering (IXS) revealed a transition from sp^2 carbon (that of native C_{60}) to sp^3 carbon. Together, these data pro-

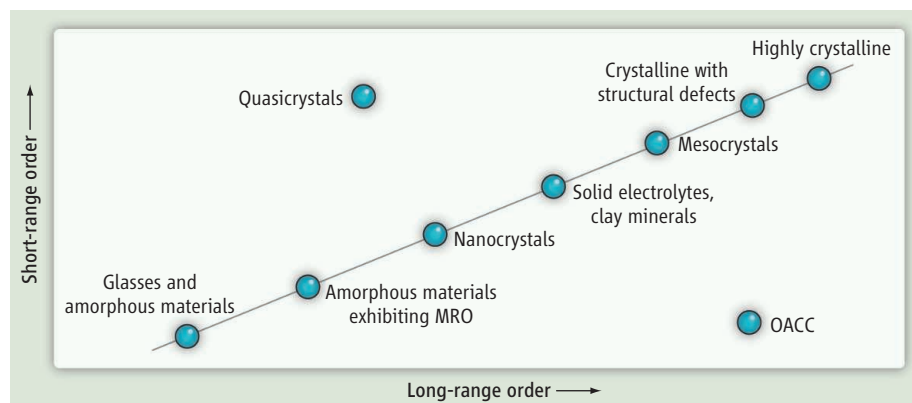
vide strong evidence of a chemically heterogeneous and structurally disordered system, which could be termed amorphous. However, the *m*-xylene organic spacer that solvates the surfaces of the disordered carbon clusters is not highly reactive under the high pressures of the experiments and likely remains spatially ordered.

A simple classification of the degree of static topological order in solid-state materials can be established by quantifying their proximity to being crystalline or amorphous. The vast majority of materials exist within the continuum between these two end members (2, 3). On one side, static disorder in crystalline materials has long been of interest

The repeat units of a crystalline material can be made up of disordered clusters.

because of the need to control defects in engineered materials for various applications. Crystallographic defects such as vacancies, interstitials, or stacking faults decrease the coherence of the crystal lattice, especially in crystalline nanomaterials, where a large number of atoms are at the interface resulting in surface relaxation effects that further reduce the size of their coherent domains (4).

Another example of crystalline solids showing a high degree of crystallographic disorder (i.e., disorder relative to a defect-free periodic lattice) is the cationic disorder. In solid electrolytes, such as Ag_2S or AgI , silver cations form a sublattice that exhibits a high degree of disorder, which results in a



Order up. Short-range order in most materials shows a strong dependence on long-range topological static order or disorder. Wang *et al.* show that the formation of OACC enlarges our current knowledge of this order-disorder diagram by expanding it to a region where amorphous clusters can be arranged in a periodic lattice, and it may serve as a conceptual end member to quasicrystals, which show substantial short-range ordering but little topological static ordering. In OACC, weak interacting solvent molecules of *m*-xylene act as spacers of the amorphous carbon clusters and hold the periodic structure.

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diffraction pattern with both amorphous and crystalline characteristics (5). In clay minerals, cations occupy positions in the interlayer pores that compensate the negative structural charge generated by isomorphic substitutions randomly distributed within the solid framework (6).

On the other end, although amorphous solids do not typically show long-range order, they can sometimes show hints of medium-range order (MRO) at the nanometer scale, especially when strong interatomic interactions are present (i.e., covalent solids) (7). In some of these materials, the “stiffness” and stereochemical (or directed) character of the intramolecular bonds is translated into a certain pseudo-periodicity at the medium-range scale that is reflected by the presence of a prepeak or first sharp diffraction peak in the x-ray or neutron diffraction pattern. Possible origins for MRO include the presence of interstitial-void correlations, the formation of ring-structure topologies, cation-cation correlations, or density fluctuations [see Zaug *et al.* (8) and references therein].

How do the findings of Wang *et al.* integrate into our current understanding of statically ordered or disordered materials (see the figure)? Generally, highly crystalline materials form through crystal-growth processes near equilibrium in the absence of impurities, or they are annealed at high temperatures in order to reach low-energy topological configurations. Under these conditions, growth units attach without structural distortion. At conditions further from equilibrium or in the presence of impurities, structural distortions (defects) result in higher degrees of structural disorder but the materials is still crystalline.

However, amorphous materials are synthesized by introducing substantial levels of impurities or by rapid formation during a phase transition, commonly melt quenching or solution precipitation, under conditions far from equilibrium. When disorder is induced during a phase transition, it extends to the molecular or atomic scale and necessarily propagates to longer length scales. When such materials are formed, it is likely impossible to introduce structural coherency over longer length scales, resulting in materials with hints of MRO at best.

Another mode of building order is the recently reported formation of mesocrystals (9) and the occurrence of oriented attachment pathways (10, 11). Under conditions far from equilibrium, nanoparticles nucleate but grow so slowly that they aggregate in a structurally coherent manner. However, long-range order is hindered by low-angle misalignment between nanoparticles.

Another unusual mode of building materials is found in quasicrystals, where metals can precipitate with high degrees of short-range rotational symmetry ordering that lack translational symmetry, resulting in poor long-range ordering.

The formation of OACC generates partial disorder through a secondary process, in this case pressure-induced amorphization, where the C_{60} component is deformable but the *m*-xylene is not. This approach could be generalized to produce hybrid structures, where one component in a multicomponent system could be designed to deform and become amorphous. The presence of a weak interacting spacer molecule seems to be an important requirement in order to keep crystallinity in the final deformed system. This work opens the door for the synthesis of intercalated sp^3 amorphous carbon materials with the potential for a huge variety of physical properties. For example, if dopant molecules replaced the *m*-xylene space, it might be possible to combine the superconductivity in alkali metal-intercalated C_{60}

(fullerides) (12) and the exceptional high hardness (13) in sp^3 three-dimensional polymers of C_{60} .

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CELL BIOLOGY

An Ancient Portal to Proteolysis

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The discovery in archaea of an alternative proteasome based on Cdc48 provides insights into the evolution of protein degradation machines.

Selective protein degradation in eukaryotes is mediated primarily by the ubiquitin-proteasome system, in which the small protein ubiquitin is covalently attached to a target protein to signal its degradation by the 26S proteasome (1). The ubiquitin-proteasome system may include as many as 1000 distinct gene products, thus constituting one of the broadest regulatory systems in nature. Recent work, including a report by Barthelme and Sauer on page 843 of this issue (2), has shed light on how this baroque pathway may have evolved and raises some unexpected possibilities for the mechanism by which proteins are delivered to proteasomes for their destruction.

Although no prokaryote has a bona fide version of the eukaryotic ubiquitin-protea-

somes pathway, various archaea and bacteria contain adenosine triphosphate (ATP)-dependent proteases that are of the same basic design (3, 4). For example, many archaea have a proteasome-like complex known as PAN (3, 4). PAN is composed of a hollow, 28-subunit proteolytic core particle (the 20S core particle), in which proteins are degraded, and a 6-subunit adenosine triphosphatase (ATPase) complex (the PAN cap). The PAN cap activates the core particle by opening up a channel and translocating in substrates while unfolding their globular domains. Ancestral variants of ubiquitin are also found in archaea, although the most prominent of these are not used to tag proteins for degradation (5). Nonetheless, ubiquitination-like protein conjugation systems linked to protein degradation have been found in archaea and some bacteria (6, 7).

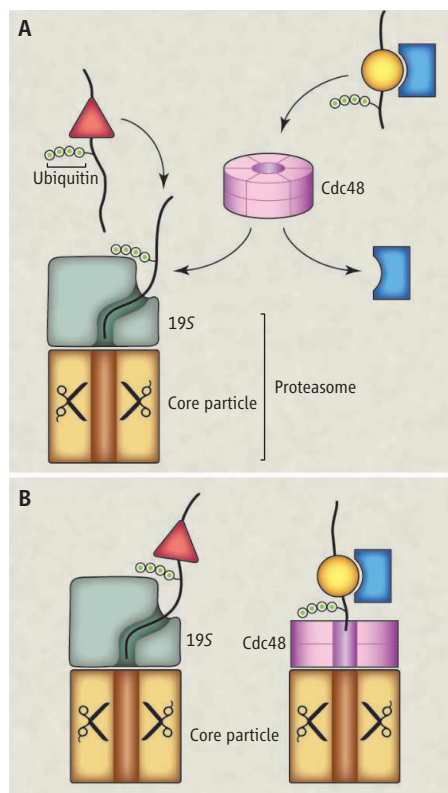
Among the curiosities of the eukaryotic ubiquitin-proteasome system is an ATPase complex known as Cdc48 (sometimes called p97 or VCP) (8). Its subunits form a six-membered ring, similar to the proteasomal

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ATPases, but their amino acid sequences are only distantly related. Cdc48 is involved in a range of cellular functions that involve ubiquitin but not always protein degradation. Mechanistically, Cdc48 appears to work in two different modes. One is to serve as a ubiquitin chain editing platform by interacting with factors that recognize ubiquitinated substrates, and with enzymes that add or remove ubiquitin (8, 9). The other is to extract ubiquitinated proteins from membranes or complex structures such as chromatin and deliver them to proteasomes in the cytosol (8, 10, 11). Thus, Cdc48 seems to prepare substrates for the proteasome in various ways—handing them to the proteasome's ATPase caps (called 19S caps in eukaryotes), which then feed them into the proteolytic core. Oddly, the 19S caps (1) have functions similar to Cdc48. They also bind to ubiquitin and to enzymes that add or remove ubiquitin, and they can unfold stable proteins and disassemble complexes (12). Could a substrate bypass the 19S caps on the way to degradation?

The C terminus of Cdc48 contains the distinctive HbYX motif (2), which had been identified in archaeal PAN (3) and was shown to mediate PAN's binding to the core particle. The motif is also highly conserved in the eukaryotic 19S caps (2, 3), where it again mediates interaction with the core particle. Thus, its appearance also in Cdc48 has been a persistent mystery. To address this, Barthelme and Sauer astutely focused on archaea rather than eukaryotes. They noticed that the core particle is found in all archaea but that PAN is missing in some, which suggested the presence of an unidentified ATPase cap. Given the HbYX motifs of Cdc48, they tested whether archaeal Cdc48 could be this missing cap. Indeed, archaeal Cdc48 bound the core particle, predominantly through the HbYX motif, and was capable of injecting substrates into the core particle cavity.

What do these findings tell us about the origin of the eukaryotic protein degradation system and its overall design? There are two principal interpretations (see the figure). The more conservative model 1 posits that in archaea, PAN and Cdc48 function in parallel as core particle activators. As the eukaryotic system evolved, Cdc48 was pushed aside to function upstream of the proteasome rather than as a part of it, and no longer docks onto the core particle. In this model, Cdc48 became deeply entrenched in the ubiquitin pathway even as it lost its direct connection to the core particle, as evidenced by its association with a large number of ubiquitin receptors that bring substrates to it (8, 13). Also, many proteins require Cdc48 for deg-



Two models of eukaryotic Cdc48 action. (A) In model 1, Cdc48 has lost its ancient ability to interact directly with the core particle and functions as an accessory factor to deliver substrates to the 26S proteasome, which contains its own 19S caps. Cdc48 can disassemble protein complexes and serve as a ubiquitin editing platform. **(B)** In model 2, Cdc48 retains its ability to associate with the proteasome core particle, and this allows two types of activated proteasome complexes with distinct ATPase caps to form. The different caps may be optimized for different subsets of proteasome substrates. Substrate interaction with the two proteasome particles in both models can be mediated by adaptors, which are not shown in the figure.

radation (8). This model fails to account for the conservation of Cdc48's HbYX motifs in eukaryotes, although there may be alternative explanations for this (14, 15). Moreover, it is puzzling that an enzyme complex pre-adapted to degrade ubiquitin conjugates in the eukaryotic lineage would have been passed over for this role. A possible rationale would be that, as Cdc48 evolved into a broadly acting factor for extracting proteins from membranes and complexes, tight coupling to the core particle and thus degradation became a liability.

Model 2 asserts that Cdc48 functions similarly in both eukaryotes and archaea. Thus, in eukaryotes, the 19S cap and Cdc48 work in parallel to deliver substrates to the proteolytic core particle. This has good logic but conflicts with the long-standing paradigm of Cdc48 working upstream of the fully assembled

proteasome (8). First, no eukaryotic Cdc48–core particle complex has ever been isolated or successfully reconstituted in eukaryotes, and not for a lack of trying. To identify an interaction between Cdc48 and the core particle in archaea, Barthelme and Sauer used an ATPase-defective mutant of Cdc48 because ATP hydrolysis weakens contacts at the Cdc48–core particle interface. Thus, the same trick may be necessary to detect an interaction in the eukaryotic system. The eukaryotic 26S proteasome is quite stable, but there is ample precedent for instability of activator–core particle complexes in bacteria. The second caveat is that, to our knowledge, no protein has been reported to require Cdc48 but not the 19S cap for proteasomal degradation. However, surprisingly few substrates have been tested for this (11), and perhaps the right proteins have not yet been examined.

If the 19S and Cdc48 caps both recognize substrates through ubiquitin, how might their functions differ? One possibility is that the Cdc48 and 19S caps unfold proteins by different mechanisms, such that each cap is best suited to unfolding a distinct subset of protein folds. Alternatively, the difference could lie in the step that follows the first recognition as the proteasome engages its substrates at initiation sequences. For example, in the 19S caps, the entrance to the degradation channel is somewhat occluded (16) so that it might be inaccessible to some sequences (17).

The potential new function of Cdc48 as a proteasome component would provide Cdc48 with a clearer role in the ubiquitin pathway and tighten the pathway's overall design. However, conceptual neatness has never been a good predictor of biology.

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Type 6 Secretion Dynamics Within and Between Bacterial Cells

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The bacterial type 6 secretion system (T6SS) is a dynamic apparatus that translocates proteins between cells by a mechanism analogous to phage tail contraction (1–3). In *Vibrio cholerae*, two proteins (VipA and VipB) build a phage tail sheathlike tubular structure in the cytosol of “predator” cells that is either extended or contracted (3). Contraction of the extended VipA/VipB sheath is thought to drive the T6SS spike and inner tube complex out of the effector or predator cell and into an adjacent target or “prey” cell (3). Disassembly of the cytoplasmic contracted sheath requires ClpV in vivo (3), a AAA+ adenosine triphosphatase (ATPase) that binds VipA/VipB tubules in vitro and can remodel these structures in the presence of ATP (4, 5). In *Pseudomonas aeruginosa*, ClpV1–green fluorescent protein (GFP) localizes to discrete foci that depend on T6SS function (6).

We used time-lapse fluorescence microscopy to follow ClpV localization in live *V. cholerae* 2740-80 cells. Functional ClpV–super folder GFP (sfGFP) and mCherry2 fusion proteins assembled at random times into short structures that disappeared in tens of seconds. In the Δ VipA background, ClpV was evenly distributed in cytosol, suggesting that the short ClpV structures were dependent on T6SS sheaths (fig. S1 and movie S1). We used functional VipA–sfGFP (3) and ClpV–mCherry2 fusions to image ClpV and T6SS sheaths simultaneously. Extended VipA–sfGFP-containing sheaths were not colocalized with ClpV–mCherry2,

whereas contraction of a sheath led to immediate colocalization of ClpV–mCherry2 with the whole contracted sheath (Fig. 1 and movies S2 and S3). Half of the ClpV associated with the contracted sheath between 683 and 1273 ms (average = 952 ms, SD = 164 ms, $n = 10$; fig. S2 and movie S4). The disassembly of the contracted sheath required between 22 and 46 s (average = 32.5 s, SD = 6.1 s, $n = 40$), measured from the moment of contraction to the moment when both ClpV and VipA signals were no longer colocalized to one spot (movies S2 and S3).

The Tyr⁶⁶⁴→Ala⁶⁶⁴ (Y664A) mutation in the pore of ClpV blocks VipA/VipB disassembly but still allows binding of ClpV to VipB in vitro (4) whereas the Phe⁸⁷→Arg⁸⁷ (F87R) mutation of N-terminal domain of ClpV blocks VipB recognition in vitro (5). In vivo, ClpV–Y664A–mCherry2 was colocalized with VipA–sfGFP to short non-dynamic structures that were likely contracted T6SS sheaths (fig. S3 and movie S5). In contrast, ClpV–F87R–mCherry2 was distributed uniformly in the cytosol with only VipA–sfGFP localized into contracted nondynamic sheaths (fig. S3 and movie S5). Localization of these ClpV mutants and the change in the dynamics of VipA-containing structures are consistent with published data (4, 5) and suggest that, in vivo, the N terminus of VipB is exposed on the surface of the contracted sheath just before its disassembly.

In *P. aeruginosa*, mutation of the regulatory gene *retS* allows expression of one of its T6SS loci (6). To assess the dynamics of T6SS in *P. aeruginosa*, we imaged a ClpV1–GFP fusion protein in a *retS* mutant (6) by time-lapse fluorescence microscopy. In contrast to *V. cholerae*, only a subset of *P. aeruginosa* cells actively formed and disassembled ClpV1–GFP-containing complexes during the observation period; the formation and dynamics of these structures required the VipA homolog PA0083 (fig. S1 and movies S6 and S7). ClpV1–GFP structures often assembled and disassembled repeatedly in apparently the same subcellular location (Fig. 1D and movie S7) indicating that, in contrast to *V. cholerae* (3), multiple T6SS apparatuses assemble in close proximity or, more likely, T6SS “base plate” components (3) are recycled by *P. aeruginosa*.

P. aeruginosa cells responded to T6SS activity occurring in a neighboring sister cell with an increase in their own T6SS dynamics (Fig. 1D and movies S6 and S7). Over time, the coincidence of T6SS activity between pairs of sister cells (termed T6SS dueling) became the dominant category

of T6SS activity observable in the *P. aeruginosa* population (table S1 and fig. S4). Spatially concurrent T6SS activity could not be documented between *V. cholerae* sister cells because this species exhibited much higher levels of T6SS activity in nearly all cells (fig. S4). The spatial and temporal coincidence of T6SS activity in adjacent *P. aeruginosa* cells strongly suggests that a signal was being transferred between cells precisely at the position of the initial T6SS activity. The *P. aeruginosa* T6SS is thought to transfer peptidoglycan-hydrolyzing T6SS substrates into sister cells that express immunity proteins to their action (7). Perhaps cellular attack by a T6SS apparatus mediated by translocation of T6SS components (e.g., the spike/inner tube complex or effector proteins) into nearby adjacent sister cells induces local cell-envelope alterations [e.g., membrane perturbation, mild peptidoglycan hydrolysis, or protein phosphorylation (7, 8)] that trigger the formation of a T6SS apparatus in the vicinity of such alterations (fig. S5).

ClpV imaging provides evidence that *P. aeruginosa* likely recycles T6SS membrane base plate components and can sense T6SS activity in nearby cells. Because T6SS dueling events were spatially and temporally linked, they likely mark the exact location of T6SS translocation of protein components (e.g., VgrG and/or effector proteins) between cells. T6SS dueling may reflect social interactions between heterologous T6SS⁺ species that coexist in the same niche.

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Supplementary Materials

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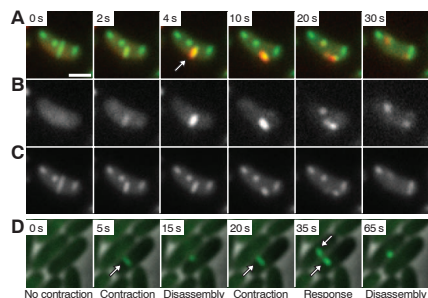


Fig. 1. ClpV colocalizes with contracted sheath (arrows). A 3-μm-by-3-μm field of cells is shown. Scale bar in (A) is 1 μm and applies to (A) to (D). (A to C) *V. cholerae* ClpV–mCherry2 + pBAD24–VipA–sfGFP. (A) Merge of ClpV–mCherry2 and VipA–sfGFP signals. (B) ClpV–mCherry2 signal. (C) VipA–sfGFP signal. Additional frames and cells are shown in movies S2 and S3. (D) *P. aeruginosa* Δ retS/ClpV1–GFP; additional frames and cells are shown in movies S6 and S7.

A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity

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Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems provide bacteria and archaea with adaptive immunity against viruses and plasmids by using CRISPR RNAs (crRNAs) to guide the silencing of invading nucleic acids. We show here that in a subset of these systems, the mature crRNA that is base-paired to trans-activating crRNA (tracrRNA) forms a two-RNA structure that directs the CRISPR-associated protein Cas9 to introduce double-stranded (ds) breaks in target DNA. At sites complementary to the crRNA-guide sequence, the Cas9 HNH nuclease domain cleaves the complementary strand, whereas the Cas9 RuvC-like domain cleaves the noncomplementary strand. The dual-tracrRNA:crRNA, when engineered as a single RNA chimera, also directs sequence-specific Cas9 dsDNA cleavage. Our study reveals a family of endonucleases that use dual-RNAs for site-specific DNA cleavage and highlights the potential to exploit the system for RNA-programmable genome editing.

Bacteria and archaea have evolved RNA-mediated adaptive defense systems called clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) that protect organisms from invading viruses and plasmids (1–3). These defense systems rely on small RNAs for sequence-specific detection and silencing of foreign nucleic acids. CRISPR/Cas systems are composed of *cas* genes organized in operon(s) and CRISPR array(s) consisting of genome-targeting sequences (called spacers) interspersed with identical repeats (1–3). CRISPR/Cas-mediated immunity occurs in three steps. In the adaptive phase, bacteria and archaea harboring one or more CRISPR loci respond to viral or plasmid challenge by integrating short fragments of foreign sequence (protospacers) into the host chromosome at the proximal end of the CRISPR array (1–3). In the expression and interference phases, transcription of the repeat-spacer element into precursor CRISPR RNA (pre-crRNA) molecules followed by enzymatic

cleavage yields the short crRNAs that can pair with complementary protospacer sequences of invading viral or plasmid targets (4–11). Target recognition by crRNAs directs the silencing of the foreign sequences by means of Cas proteins that function in complex with the crRNAs (10, 12–20).

There are three types of CRISPR/Cas systems (21–23). The type I and III systems share some overarching features: specialized Cas endonucleases process the pre-crRNAs, and once mature, each crRNA assembles into a large multi-Cas protein complex capable of recognizing and cleaving nucleic acids complementary to the crRNA. In contrast, type II systems process pre-crRNAs by a different mechanism in which a trans-activating crRNA (tracrRNA) complementary to the repeat sequences in pre-crRNA triggers processing by the double-stranded (ds) RNA-specific ribonuclease RNase III in the presence of the Cas9 (formerly Csn1) protein (fig. S1) (4, 24). Cas9 is thought to be the sole protein responsible for crRNA-guided silencing of foreign DNA (25–27).

We show here that in type II systems, Cas9 proteins constitute a family of enzymes that require a base-paired structure formed between the activating tracrRNA and the targeting crRNA to cleave target dsDNA. Site-specific cleavage occurs at locations determined by both base-pairing complementarity between the crRNA and the target protospacer DNA and a short motif [referred to as the protospacer adjacent motif (PAM)] juxtaposed to the complementary region in the target DNA. Our study further demonstrates that the Cas9 endonuclease family can be programmed with single RNA molecules to cleave specific DNA sites, thereby raising the exciting possibility of

developing a simple and versatile RNA-directed system to generate dsDNA breaks for genome targeting and editing.

Cas9 is a DNA endonuclease guided by two RNAs. Cas9, the hallmark protein of type II systems, has been hypothesized to be involved in both crRNA maturation and crRNA-guided DNA interference (fig. S1) (4, 25–27). Cas9 is involved in crRNA maturation (4), but its direct participation in target DNA destruction has not been investigated. To test whether and how Cas9 might be capable of target DNA cleavage, we used an overexpression system to purify Cas9 protein derived from the pathogen *Streptococcus pyogenes* (fig. S2, see supplementary materials and methods) and tested its ability to cleave a plasmid DNA or an oligonucleotide duplex bearing a protospacer sequence complementary to a mature crRNA, and a bona fide PAM. We found that mature crRNA alone was incapable of directing Cas9-catalyzed plasmid DNA cleavage (Fig. 1A and fig. S3A). However, addition of tracrRNA, which can pair with the repeat sequence of crRNA and is essential to crRNA maturation in this system, triggered Cas9 to cleave plasmid DNA (Fig. 1A and fig. S3A). The cleavage reaction required both magnesium and the presence of a crRNA sequence complementary to the DNA; a crRNA capable of tracrRNA base pairing but containing a noncognate target DNA-binding sequence did not support Cas9-catalyzed plasmid cleavage (Fig. 1A; fig. S3A, compare crRNA-sp2 to crRNA-sp1; and fig. S4A). We obtained similar results with a short linear dsDNA substrate (Fig. 1B and fig. S3, B and C). Thus, the trans-activating tracrRNA is a small noncoding RNA with two critical functions: triggering pre-crRNA processing by the enzyme RNase III (4) and subsequently activating crRNA-guided DNA cleavage by Cas9.

Cleavage of both plasmid and short linear dsDNA by tracrRNA:crRNA-guided Cas9 is site-specific (Fig. 1, C to E, and fig. S5, A and B). Plasmid DNA cleavage produced blunt ends at a position three base pairs upstream of the PAM sequence (Fig. 1, C and E, and fig. S5, A and C) (26). Similarly, within short dsDNA duplexes, the DNA strand that is complementary to the target-binding sequence in the crRNA (the complementary strand) is cleaved at a site three base pairs upstream of the PAM (Fig. 1, D and E, and fig. S5, B and C). The noncomplementary DNA strand is cleaved at one or more sites within three to eight base pairs upstream of the PAM. Further investigation revealed that the noncomplementary strand is first cleaved endonucleolytically and subsequently trimmed by a 3'-5' exonuclease activity (fig. S4B). The cleavage rates by Cas9 under single-turnover conditions ranged from 0.3 to 1 min⁻¹, comparable to those of restriction endonucleases (fig. S6A), whereas incubation of wild-type (WT) Cas9-tracrRNA:crRNA complex with a fivefold molar excess of substrate DNA provided evidence that the dual-RNA-guided Cas9 is a multiple-turnover enzyme (fig. S6B). In

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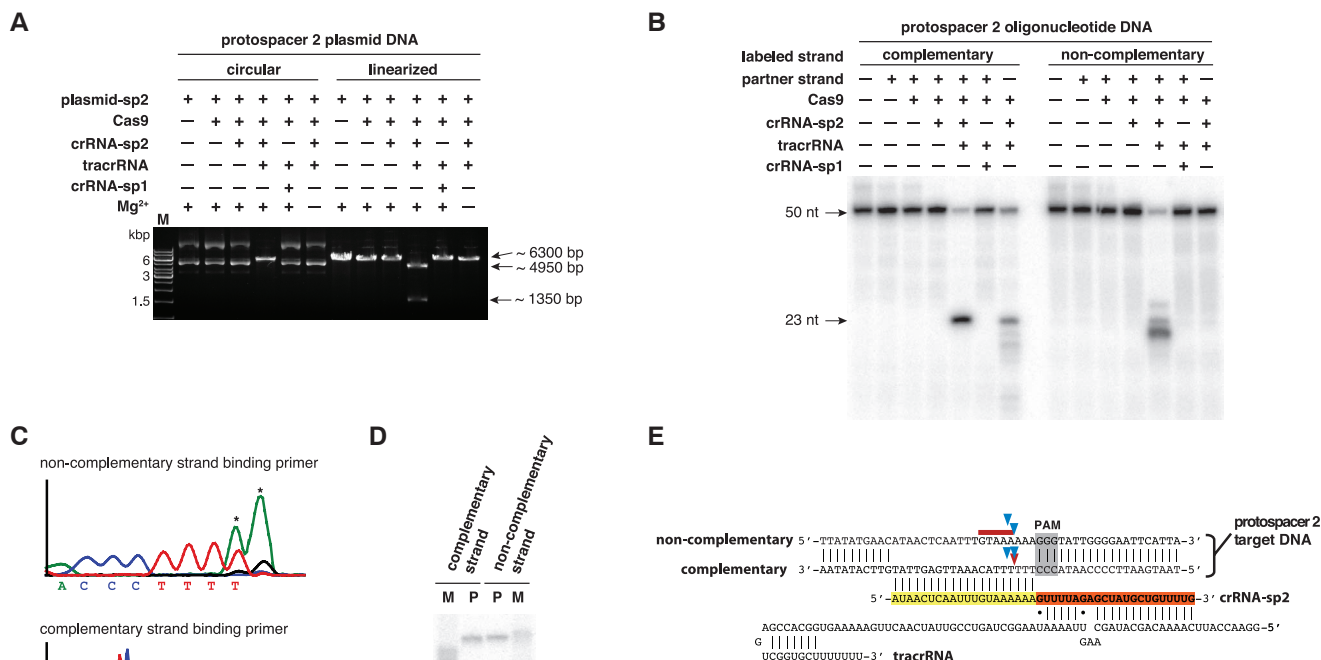


Fig. 1. Cas9 is a DNA endonuclease guided by two RNA molecules. **(A)** Cas9 was programmed with a 42-nucleotide crRNA-sp2 (crRNA containing a spacer 2 sequence) in the presence or absence of 75-nucleotide tracrRNA. The complex was added to circular or XhoI-linearized plasmid DNA bearing a sequence complementary to spacer 2 and a functional PAM. crRNA-sp1, specificity control; M, DNA marker; kbp, kilo-base pair. See fig. S3A. **(B)** Cas9 was programmed with crRNA-sp2 and tracrRNA (4). The complementary or noncomplementary strands of the DNA were 5'-radiolabeled and annealed with a nonlabeled partner strand. nt, nucleotides. See fig. S3, B and C. **(C)** Sequencing analysis of cleavage products from Fig. 1A. Termination of primer extension in the sequencing reaction indicates the position of the cleavage site. The 3' terminal A overhang (asterisks) is an artifact of the sequencing reaction. See fig. S5, A and C. **(D)** The cleavage products from Fig. 1B were analyzed alongside 5' end-labeled size markers derived from the complementary and noncomplementary strands of the target DNA duplex. M, marker; P, cleavage product. See fig. S5, B and C. **(E)** Schematic representation of tracrRNA, crRNA-sp2, and protospacer 2 DNA sequences. Regions of crRNA complementarity to tracrRNA (orange) and the protospacer DNA (yellow) are represented. The PAM sequence is shown in gray; cleavage sites mapped in (C) and (D) are represented by blue arrows (C), a red arrow [(D), complementary strand], and a red line [(D), noncomplementary strand].

contrast to the CRISPR type I Cascade complex (18), Cas9 cleaves both linearized and supercoiled plasmids (Figs. 1A and 2A). Therefore, an invading plasmid can, in principle, be cleaved multiple times by Cas9 proteins programmed with different crRNAs.

Each Cas9 nuclease domain cleaves one DNA strand. Cas9 contains domains homologous to both HNH and RuvC endonucleases (Fig. 2A and fig. S7) (21–23, 27, 28). We designed and purified Cas9 variants containing inactivating point mutations in the catalytic residues of either the HNH or RuvC-like domains (Fig. 2A and fig. S7) (23, 27). Incubation of these variant Cas9 proteins with native plasmid DNA showed that dual-RNA-guided mutant Cas9 proteins yielded nicked open circular plasmids, whereas the WT Cas9 protein-tracrRNA:crRNA complex produced a linear DNA product (Figs. 1A and 2A and figs. S3A and S8A). This result indicates that the Cas9 HNH and RuvC-like domains each cleave one plasmid DNA strand. To determine which strand of the target DNA is cleaved by each Cas9 catalytic domain, we incubated the mutant Cas9-tracrRNA:crRNA

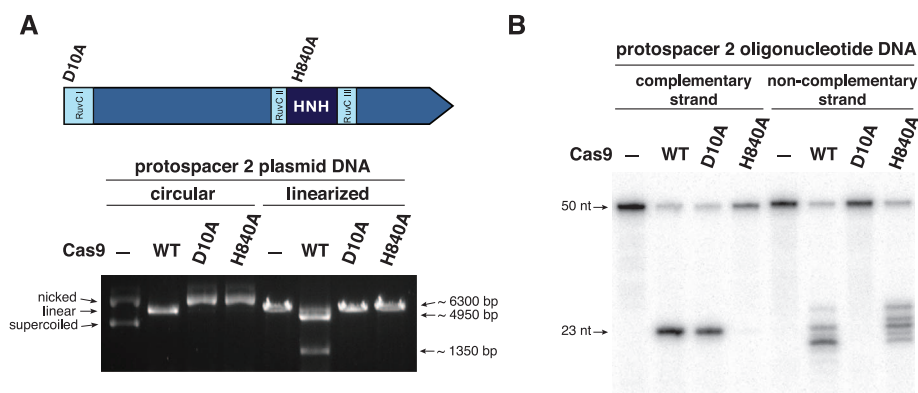


Fig. 2. Cas9 uses two nuclease domains to cleave the two strands in the target DNA. **(A)** (Top) Schematic representation of Cas9 domain structure showing the positions of domain mutations. D10A, Asp¹⁰→Ala¹⁰; H840A, His⁸⁴⁰→Ala⁸⁴⁰. (Bottom) Complexes of WT or nuclease mutant Cas9 proteins with tracrRNA:crRNA-sp2 were assayed for endonuclease activity as in Fig. 1A. **(B)** Complexes of WT Cas9 or nuclease domain mutants with tracrRNA and crRNA-sp2 were tested for activity as in Fig. 1B.

complexes with short dsDNA substrates in which either the complementary or noncomplementary strand was radiolabeled at its 5' end. The resulting cleavage products indicated that the

Cas9 HNH domain cleaves the complementary DNA strand, whereas the Cas9 RuvC-like domain cleaves the noncomplementary DNA strand (Fig. 2B and fig. S8B).

(30, 31) and the Cascade and Csy CRISPR complexes (13, 14).

A short sequence motif dictates R-loop formation. In multiple CRISPR/Cas systems, recognition of self versus nonself has been shown to involve a short sequence motif that is preserved in the foreign genome, referred to as the PAM (27, 29, 32–34). PAM motifs are only a few base pairs in length, and their precise sequence and position vary according to the CRISPR/Cas system type (32). In the *S. pyogenes* type II system, the PAM conforms to an NGG consensus sequence, containing two G:C base pairs that occur one base pair downstream of the crRNA binding sequence, within the target DNA (4). Transformation assays demonstrated that the GG motif is essential for protospacer plasmid DNA elimination by CRISPR/Cas in bacterial cells (fig. S13A), consistent with previous observations in *S. thermophilus* (27). The motif is also essential for in vitro protospacer plasmid cleavage by tracrRNA:crRNA-guided Cas9 (fig. S13B). To determine the role of the PAM

in target DNA cleavage by the Cas9-tracrRNA:crRNA complex, we tested a series of dsDNA duplexes containing mutations in the PAM sequence on the complementary or noncomplementary strands, or both (Fig. 4A). Cleavage assays using these substrates showed that Cas9-catalyzed DNA cleavage was particularly sensitive to mutations in the PAM sequence on the noncomplementary strand of the DNA, in contrast to complementary strand PAM recognition by type I CRISPR/Cas systems (18, 34). Cleavage of target single-stranded DNAs was unaffected by mutations of the PAM motif. This observation suggests that the PAM motif is required only in the context of target dsDNA and may thus be required to license duplex unwinding, strand invasion, and the formation of an R-loop structure. When we used a different crRNA-target DNA pair (crRNA-sp4 and protospacer 4 DNA), selected due to the presence of a canonical PAM not present in the protospacer 2 target DNA, we found that both G nucleotides of the PAM were required for efficient Cas9-catalyzed DNA

cleavage (Fig. 4B and fig. S13C). To determine whether the PAM plays a direct role in recruiting the Cas9-tracrRNA:crRNA complex to the correct target DNA site, we analyzed binding affinities of the complex for target DNA sequences by native gel mobility shift assays (Fig. 4C). Mutation of either G in the PAM sequence substantially reduced the affinity of Cas9-tracrRNA:crRNA for the target DNA. This finding argues for specific recognition of the PAM sequence by Cas9 as a prerequisite for target DNA binding and possibly strand separation to allow strand invasion and R-loop formation, which would be analogous to the PAM sequence recognition by CasA/Cse1 implicated in a type I CRISPR/Cas system (34).

Cas9 can be programmed with a single chimeric RNA. Examination of the likely secondary structure of the tracrRNA:crRNA duplex (Figs. 1E and 3C) suggested the possibility that the features required for site-specific Cas9-catalyzed DNA cleavage could be captured in a single chimeric RNA. Although the tracrRNA:crRNA

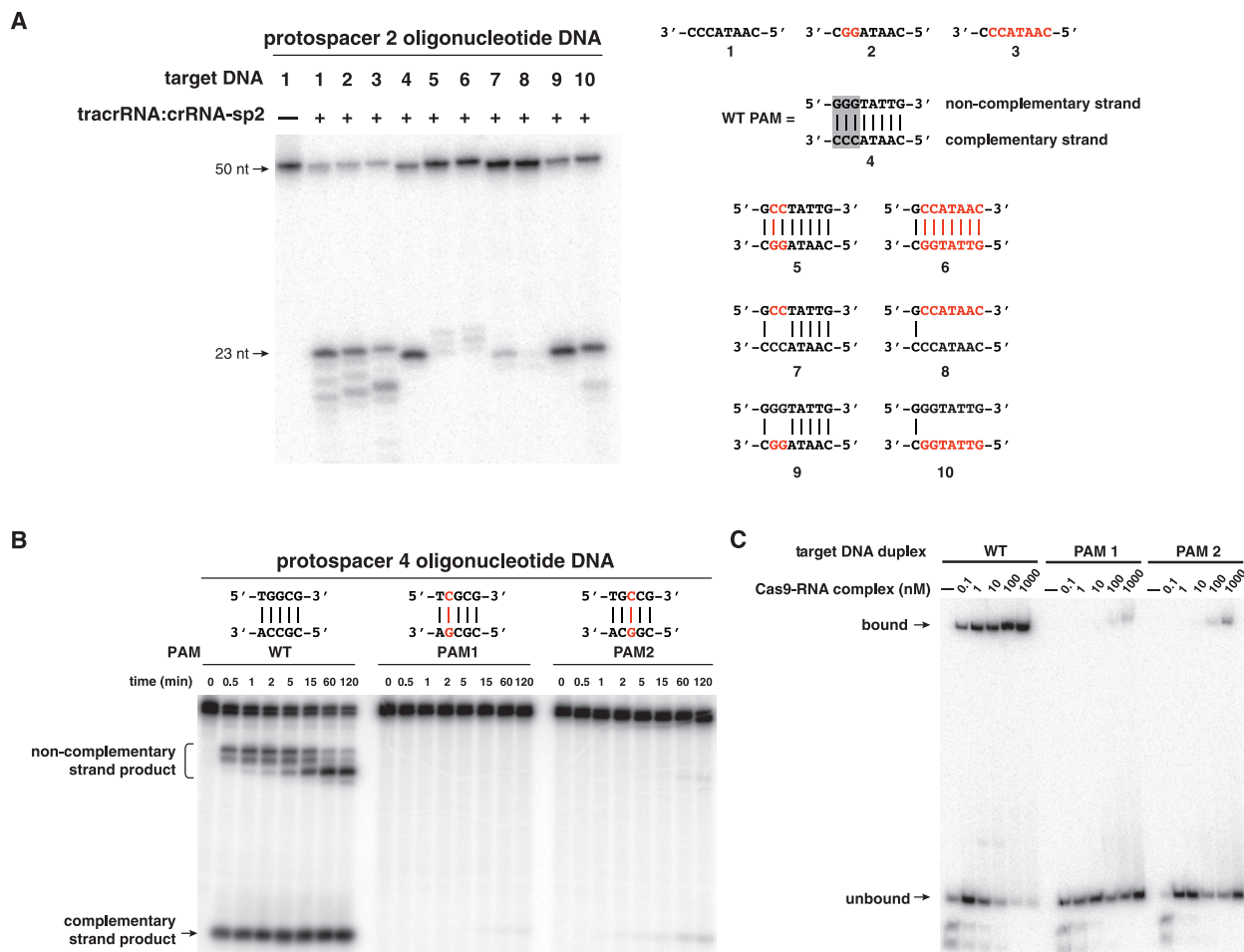


Fig. 4. A PAM is required to license target DNA cleavage by the Cas9-tracrRNA:crRNA complex. **(A)** Dual RNA-programmed Cas9 was tested for activity as in Fig. 1B. WT and mutant PAM sequences in target DNAs are indicated (right). **(B)** Protospacer 4 target DNA duplexes (labeled at both 5' ends) containing WT and mutant PAM motifs were incubated with Cas9 programmed with tracrRNA:crRNA-sp4 (nucleotides 23 to 89). At the indi-

cated time points (in minutes), aliquots of the cleavage reaction were taken and analyzed as in Fig. 1B. **(C)** Electrophoretic mobility shift assays were performed using RNA-programmed Cas9 (D10A/H840A) and protospacer 4 target DNA duplexes [same as in (B)] containing WT and mutated PAM motifs. The Cas9 (D10A/H840A)–RNA complex was titrated from 100 pM to 1 μ M.

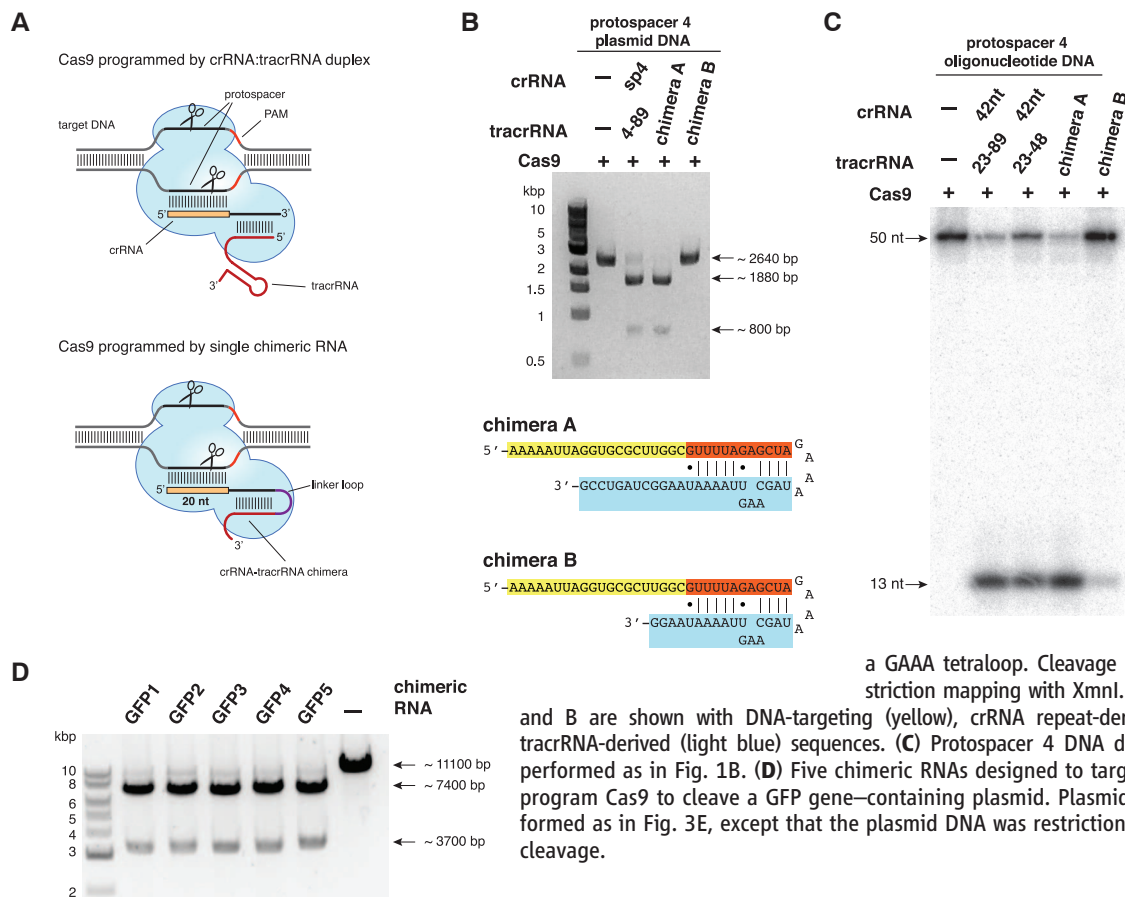


Fig. 5. Cas9 can be programmed using a single engineered RNA molecule combining tracrRNA and crRNA features. **(A)** (Top) In type II CRISPR/Cas systems, Cas9 is guided by a two-RNA structure formed by activating tracrRNA and targeting crRNA to cleave site-specifically-targeted dsDNA (see fig. S1). (Bottom) A chimeric RNA generated by fusing the 3' end of crRNA to the 5' end of tracrRNA. **(B)** A plasmid harboring protospacer 4 target sequence and a WT PAM was subjected to cleavage by Cas9 programmed with tracrRNA(4-89):crRNA-sp4 duplex or in vitro-transcribed chimeric RNAs constructed by joining the 3' end of crRNA to the 5' end of tracrRNA with

a GAAA tetraloop. Cleavage reactions were analyzed by restriction mapping with XmnI. Sequences of chimeric RNAs A and B are shown with DNA-targeting (yellow), crRNA repeat-derived sequences (orange), and tracrRNA-derived (light blue) sequences. **(C)** Protospacer 4 DNA duplex cleavage reactions were performed as in Fig. 1B. **(D)** Five chimeric RNAs designed to target the GFP gene were used to program Cas9 to cleave a GFP gene-containing plasmid. Plasmid cleavage reactions were performed as in Fig. 3E, except that the plasmid DNA was restriction mapped with AvrII after Cas9 cleavage.

target-selection mechanism works efficiently in nature, the possibility of a single RNA-guided Cas9 is appealing due to its potential utility for programmed DNA cleavage and genome editing (Fig. 5A). We designed two versions of a chimeric RNA containing a target recognition sequence at the 5' end followed by a hairpin structure retaining the base-pairing interactions that occur between the tracrRNA and the crRNA (Fig. 5B). This single transcript effectively fuses the 3' end of crRNA to the 5' end of tracrRNA, thereby mimicking the dual-RNA structure required to guide site-specific DNA cleavage by Cas9. In cleavage assays using plasmid DNA, we observed that the longer chimeric RNA was able to guide Cas9-catalyzed DNA cleavage in a manner similar to that observed for the truncated tracrRNA:crRNA duplex (Fig. 5B and fig. S14, A and C). The shorter chimeric RNA did not work efficiently in this assay, confirming that nucleotides that are 5 to 12 positions beyond the tracrRNA:crRNA base-pairing interaction are important for efficient Cas9 binding and/or target recognition. We obtained similar results in cleavage assays using short dsDNA as a substrate, further indicating that the position of the cleavage site in target DNA is identical to that observed using the dual tracrRNA:crRNA as a guide (Fig. 5C and fig. S14, B and C). Finally, to establish whether the design of chimeric RNA

might be universally applicable, we engineered five different chimeric guide RNAs to target a portion of the gene encoding the green-fluorescent protein (GFP) (fig. S15, A to C) and tested their efficacy against a plasmid carrying the GFP coding sequence in vitro. In all five cases, Cas9 programmed with these chimeric RNAs efficiently cleaved the plasmid at the correct target site (Fig. 5D and fig. S15D), indicating that rational design of chimeric RNAs is robust and could, in principle, enable targeting of any DNA sequence of interest with few constraints beyond the presence of a GG dinucleotide adjacent to the targeted sequence.

Conclusions. We identify a DNA interference mechanism involving a dual-RNA structure that directs a Cas9 endonuclease to introduce site-specific double-stranded breaks in target DNA. The tracrRNA:crRNA-guided Cas9 protein makes use of distinct endonuclease domains (HNH and RuvC-like domains) to cleave the two strands in the target DNA. Target recognition by Cas9 requires both a seed sequence in the crRNA and a GG dinucleotide-containing PAM sequence adjacent to the crRNA-binding region in the DNA target. We further show that the Cas9 endonuclease can be programmed with guide RNA engineered as a single transcript to target and cleave any dsDNA sequence of interest. The system is efficient, versatile, and programmable

by changing the DNA target-binding sequence in the guide chimeric RNA. Zinc-finger nucleases and transcription-activator-like effector nucleases have attracted considerable interest as artificial enzymes engineered to manipulate genomes (35–38). We propose an alternative methodology based on RNA-programmed Cas9 that could offer considerable potential for gene-targeting and genome-editing applications.

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Supplementary Materials

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Materials and Methods
Figs. S1 to S15
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REPORTS

Long-Range Incommensurate Charge Fluctuations in (Y,Nd)Ba₂Cu₃O_{6+x}

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The concept that superconductivity competes with other orders in cuprate superconductors has become increasingly apparent, but obtaining direct evidence with bulk-sensitive probes is challenging. We have used resonant soft x-ray scattering to identify two-dimensional charge fluctuations with an incommensurate periodicity of ~ 3.2 lattice units in the copper-oxide planes of the superconductors (Y,Nd)Ba₂Cu₃O_{6+x} with hole concentrations of 0.09 to 0.13 per planar Cu ion. The intensity and correlation length of the fluctuation signal increase strongly upon cooling down to the superconducting transition temperature (T_c); further cooling below T_c abruptly reverses the divergence of the charge correlations. In combination with earlier observations of a large gap in the spin excitation spectrum, these data indicate an incipient charge density wave instability that competes with superconductivity.

A successful theory of high-temperature superconductivity in the copper oxides requires a detailed understanding of the spin, charge, and orbital correlations in the normal state from which superconductivity emerges.

In recent years, evidence of ordering phenomena in which these correlations might take on particularly simple forms has emerged (1, 2). Despite intense efforts, however, only two order parameters other than superconductivity have thus far been unambiguously identified by bulk-sensitive experimental probes: (i) uniform antiferromagnetism in undoped insulating cuprates and (ii) uniaxially modulated antiferromagnetism (3) combined with charge order (3, 4) in doped cuprates of the so-called “214” family [that is, compounds of composition La_{2-x-y}(Sr,Ba)_x(Nd,Eu)_yCuO₄]. The latter is known as “stripe order,” with a commensurate charge modulation of period $4a$ (where lattice unit $a = 3.8$ to 3.9 Å is the distance between neighboring Cu atoms in the CuO₂ planes), which greatly reduces the superconducting transition temperature (T_c) of 214 materials at a doping level $p \sim 1/8$ per planar Cu atom. Incommensurate spin fluctuations in 214 materials with $p \neq 1/8$

(5) have been interpreted as evidence of fluctuating stripes (6). A long-standing debate has evolved around the questions of whether stripe order is a generic feature of the copper oxides and whether stripe fluctuations are essential for superconductivity.

Recent attention has focused on the “123” family [RBa₂Cu₃O_{6+x} with $R = Y$ or another rare earth element], which exhibits substantially lower chemical disorder and higher maximal T_c than the 214 system. For underdoped 123 compounds, the anomaly in the T_c -versus- p relation at $p = 1/8$ (7) and the large in-plane anisotropies in the transport properties (8, 9) have been interpreted as evidence of stripe order or fluctuations, in analogy to stripe-ordered 214 materials (10). Differences in the spin dynamics of the two families have, however, cast some doubt on this interpretation. In particular, neutron-scattering studies of moderately doped 123 compounds have revealed a gap of magnitude ≥ 20 meV in the magnetic excitation spectrum (11–14), whereas 214 compounds with similar hole concentrations exhibit nearly gapless spin excitations (5). Further questions have been raised by the recent discovery of small Fermi surface pockets in quantum oscillation experiments on underdoped 123 materials in magnetic fields large enough to weaken or obliterate superconductivity (15). Some researchers have attributed this observation to a Fermi surface reconstruction due to magnetic field-induced stripe order (10), whereas others have argued that even the high magnetic fields applied in these experiments appear incapable of closing the spin gap and that a biaxial charge modulation is required to explain the quantum oscillation data (16). Nuclear magnetic resonance (NMR) experiments have shown evidence of a magnetic field-induced uniaxial charge modulation (17), but they do not yield information about electronic fluctuations outside of a very narrow energy window of ~ 1 μeV. On the other hand, scattering experiments to determine

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the periodicity of this modulation and/or to characterize its spin or charge dynamics can currently not be performed under the extreme experimental conditions required to stabilize the high-field phase. Therefore, the nature of the leading electronic instability of doped 123 materials and its relation to superconductivity and to stripe order remain largely unknown.

Here, we report the results of resonant x-ray scattering (RXS) experiments especially designed to detect charge order and/or corresponding fluctuations on electronic energy scales. In contrast to earlier nonresonant x-ray scattering experiments on the same 123 compounds (18, 19), the use of photons with energies near the Cu L_3 absorption edge ($2p_{3/2} \rightarrow 3d$ transitions, $h\nu \approx 931$ eV) greatly enhances the sensitivity of the scattering signal to the valence electron system (20). The layered crystal structure of the 123 system (Fig. 1A) and the consequent two-dimensional (2D) scattering cross section (20) allow for easy variation of the momentum-transfer component parallel to the CuO_2 planes ($q_{\parallel}$) through rotations of the sample at a fixed scattering angle (Fig. 1, B and C). Further experimental parameters include the energy of the incident photons and their polarization (Fig. 1B). Most of the measurements were carried out with a spectrometer, and the energy transferred by the photon was monitored with a combined instrumental resolution of ~ 130 meV. Unlike in conventional RXS, we could discriminate between the elastic and quasi-elastic signal coming from charge density fluctuations and the dominant inelastic contribution (Fig. 1D) (21, 22).

Our central observation is apparent in the raw spectra of underdoped $\text{Nd}_{1-x}\text{Ba}_{2-x}\text{Cu}_3\text{O}_7$, shown in Fig. 1D as a function of both photon energy loss and $q_{\parallel}$ along the (1,0) direction, parallel to the Cu-O bonds (Fig. 1B). The inelastic portion of the spectrum comprises features around 100 to 300 meV resulting from paramagnon excitations (23) and from 1 to 3 eV due to interorbital transitions (dd excitations) (21). These features depend weakly on $q_{\parallel}$, whereas the response centered at zero energy loss exhibits a pronounced maximum at $q_{\parallel} = (0.31, 0)$. Scans around symmetry-equivalent, reciprocal-space points (20) on multiple samples (see below) have confirmed that the latter feature is generic to the 123 system and that its intensity maximum is at a wave vector distinctly different from the commensurate position (1/3,0). We will refer to this feature as a resonant elastic x-ray scattering (REXS) peak, although, as shown below, it arises from low-energy fluctuations of the valence-electron charge density in the CuO_2 planes and, hence, is not truly elastic.

The 123 structure contains copper atoms in two different crystallographic sites: (i) Cu2 atoms in the CuO_2 layers that are common to all high-temperature superconductors and (ii) Cu1 atoms in chains specific to the 123 system that act as charge reservoirs for the layers (Fig. 1A). In x-ray absorption (XAS) and RXS experiments, signals arising from Cu1 and Cu2 sites can be differentiated by virtue of their distinct absorption reso-

nance energy and photon polarization dependence (Fig. 2C) (24). The anomalous REXS peak is present only when the photon energy is tuned to the $3d^9$ configuration of Cu2 sites (Fig. 2, D and E) and, hence, can be unambiguously assigned to the CuO_2 planes. Further confirmation for this assignment comes from a comparison of $q_{\parallel}$ scans along the (0,1) and (1,0) directions (parallel and perpendicular to the chains, respectively) in untwinned $\text{YBa}_2\text{Cu}_3\text{O}_{6.6}$ crystals (Fig. 2A). The presence of equally intense REXS peaks in the two directions is incompatible with an origin in the Cu1 chains.

We now use the dependence of the RXS cross section on the polarization geometry to address the question of whether the REXS signal arises from a modulation of the charge or spin density in the CuO_2 layers. To separate these two scattering channels, we measured the scattering intensity for σ (Fig. 2A) and π polarization of the

incident beam and for the two opposite directions of $q_{\parallel}$ along (1,0) (Fig. 1C). In Fig. 2B, we compare the results to model calculations for spin-flip and non-spin-flip RXS from Cu2 sites with $3d^9(x^2 - y^2)$ ground-state symmetry (25, 26). In contrast to the 100- to 300-meV loss feature, which follows the behavior expected for spin-flip scattering as seen before in undoped and doped cuprates (22, 23), the polarization dependence of the REXS peak indicates that it arises from charge scattering.

Figure 3 provides an overview of the REXS response in a variety of different 123 samples, including both single crystals of $\text{YBa}_2\text{Cu}_3\text{O}_{6+x}$, where the doping level p is adjusted through the density and arrangement of oxygen atoms in the Cu1 chain layer, and thin films of $\text{Nd}_{1-x}\text{Ba}_{2-x}\text{Cu}_3\text{O}_7$, where the Cu1 chains are fully oxygenated and p is controlled through the Nd/Ba ratio. The presence of closely similar charge-fluctuation peaks

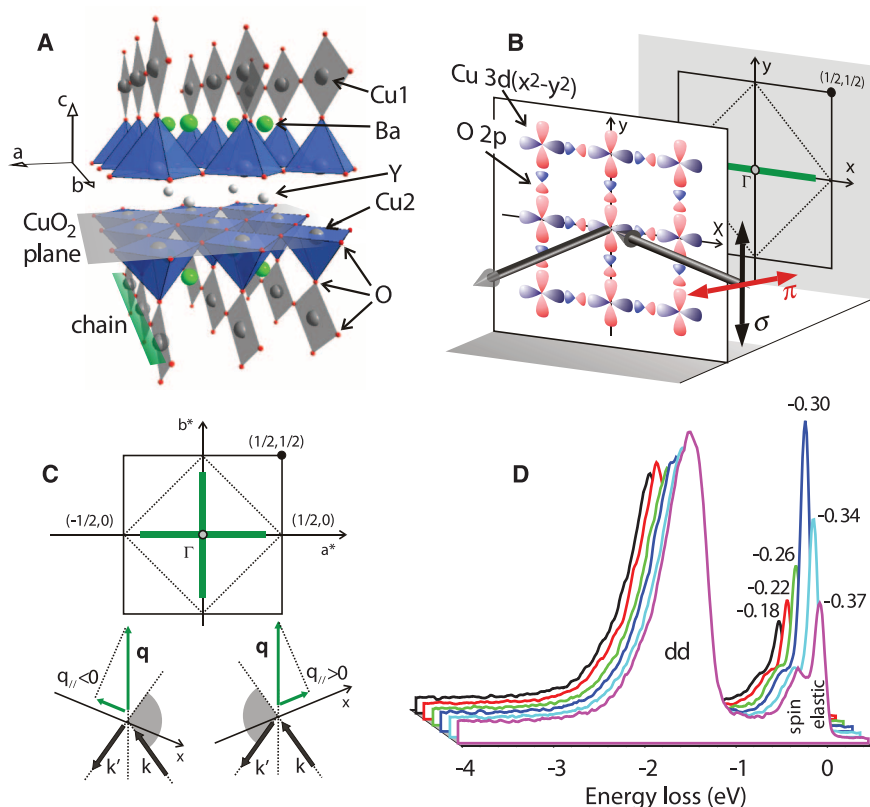


Fig. 1. Resonant soft x-ray scattering from layered cuprates. **(A)** Crystalline structure of $\text{YBa}_2\text{Cu}_3\text{O}_{6+x}$ superconductors. For $x < 1$, some of the O atoms along the chains are missing. **(B)** Scattering geometry with the c and either a or b axes in the scattering plane. The incident photon polarization can be parallel (π) or perpendicular (σ) to the scattering plane. The real and reciprocal spaces are sketched in the front and rear plane, respectively. In the real-space image, the Cu $3dx^2 - y^2$ and O $2p$ orbitals are shown. In the reciprocal-space image, the nuclear and magnetic first Brillouin zones are drawn with solid and dotted lines, respectively; the thick green line indicates the range covered by the experiments. Γ labels the (0,0) point, origin of the reciprocal space and center of the first Brillouin zone. **(C)** The in-plane component $q_{\parallel}$ of the transferred momentum ranges from -0.4 to $+0.4$ rlu along the (1,0) or (0,1) direction when the sample is rotated around the y axis at a fixed scattering angle, indicated by the gray arcs (130° in most cases). **(D)** In the Cu L_3 energy-resolved RXS spectra of underdoped $\text{Nd}_{1.2}\text{Ba}_{1.8}\text{Cu}_3\text{O}_7$ ($T_c = 65$ K, $T = 15$ K, σ polarization), the quasi-elastic component has a maximum intensity at $q_{\parallel} = -0.31$ rlu. The dispersing paramagnons (spin) are visible next to the quasi-elastic peak; the interorbital excitations (dd) around 2 eV carry most of the spectral weight.

at $|q_{||}| \approx 0.31$ reciprocal lattice units (rlu) in both systems in the range $0.09 \leq p \leq 0.13$ confirms that they are independent of the Cu1 chains and other details of the doping mechanism. Samples outside this narrow p range do not show any evidence

of the anomalous REXS response, in agreement with earlier RXS work (24). This implies weaker charge fluctuations in samples at lower p , where incommensurate magnetic order has been observed at low temperatures (27), and at higher p ,

where the superconducting T_c is maximal. The range in which the REXS peak is observed coincides with the well-known plateau in the T_c -versus- p relation of the 123 system (shaded region in Fig. 3), which suggests that competition between superconducting and charge density correlations is responsible for the anomalously low T_c in this range.

To further explore the interplay between superconductivity and the anomalous charge density response, we have measured the temperature dependence of the REXS peak, both in the energy-resolved mode presented above and in the more traditional energy-integrated detection (Fig. 4, A and B). The peak is present both above and below the superconducting T_c . It narrows continuously upon cooling toward T_c , indicating a pronounced increase of the correlation length that reaches $(16 \pm 2)a$ at T_c of $\text{YBa}_2\text{Cu}_3\text{O}_{6.6}$, to be compared with domain sizes in the range of $40a$ to $66a$ for stripe-ordered $\text{La}_{1.875}\text{Ba}_{0.125}\text{CuO}_4$ (4, 28, 29). The T -dependent correlation length demonstrates that the signal arises from charge density fluctuations, rather than a static charge density wave (CDW). The incipient CDW phase transition heralded by the nearly diverging correlation length is preempted by the superconducting transition, which manifests itself in an abrupt decrease of the intensity and correlation length of the REXS peak (Fig. 4, C and D). This constitutes direct evidence of the competition between superconducting and incommensurate CDW states, which was already suggested by the doping dependence of the REXS response (Fig. 3). CDW correlations were previously observed by scanning

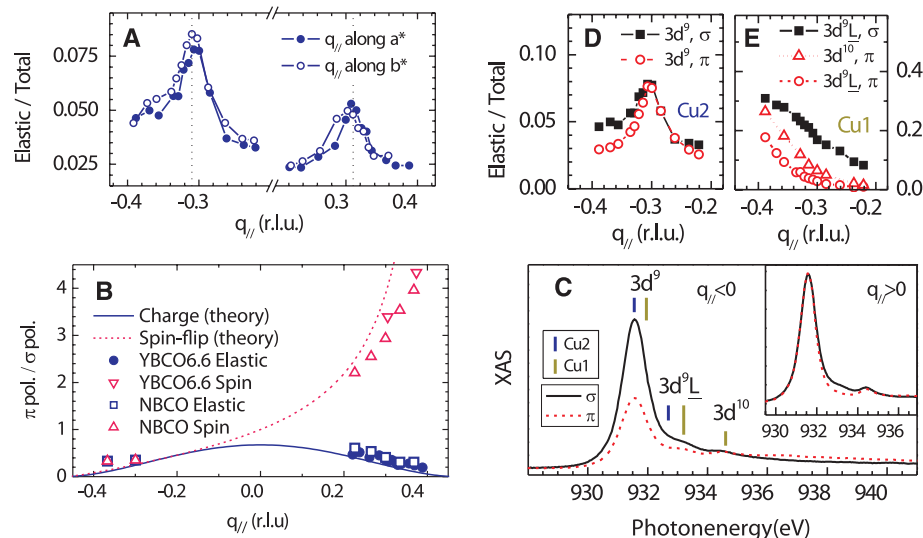


Fig. 2. Polarization and energy dependence of the REXS peak in $\text{YBa}_2\text{Cu}_3\text{O}_{6.6}$. (A) REXS scans measured at the Cu L_3 edge for positive and negative values of $q_{||}$ along (1,0) and (0,1), using σ polarization (20). (B) Ratio between the REXS signal intensities obtained with π and σ polarizations (pol.). The experimental data for $\text{YBa}_2\text{Cu}_3\text{O}_{6.6}$ and $\text{Nd}_{1.2}\text{Ba}_{1.8}\text{Cu}_3\text{O}_7$ are compared to the model calculations (see text for details) and to the magnetic excitation signal (100- to 300-meV energy loss). (C) XAS spectra of $\text{YBa}_2\text{Cu}_3\text{O}_{6.6}$ with two polarizations and two geometries corresponding to negative and positive values of $q_{||}$ (Fig. 1C). The main contributions to the XAS peaks are indicated. REXS with photon energies at the main absorption peak of 931.5 eV selects signals arising from the Cu2 sites. (D and E) REXS scans show the CDW peak only when exciting at the Cu2 sites and show nothing at higher excitation energies.

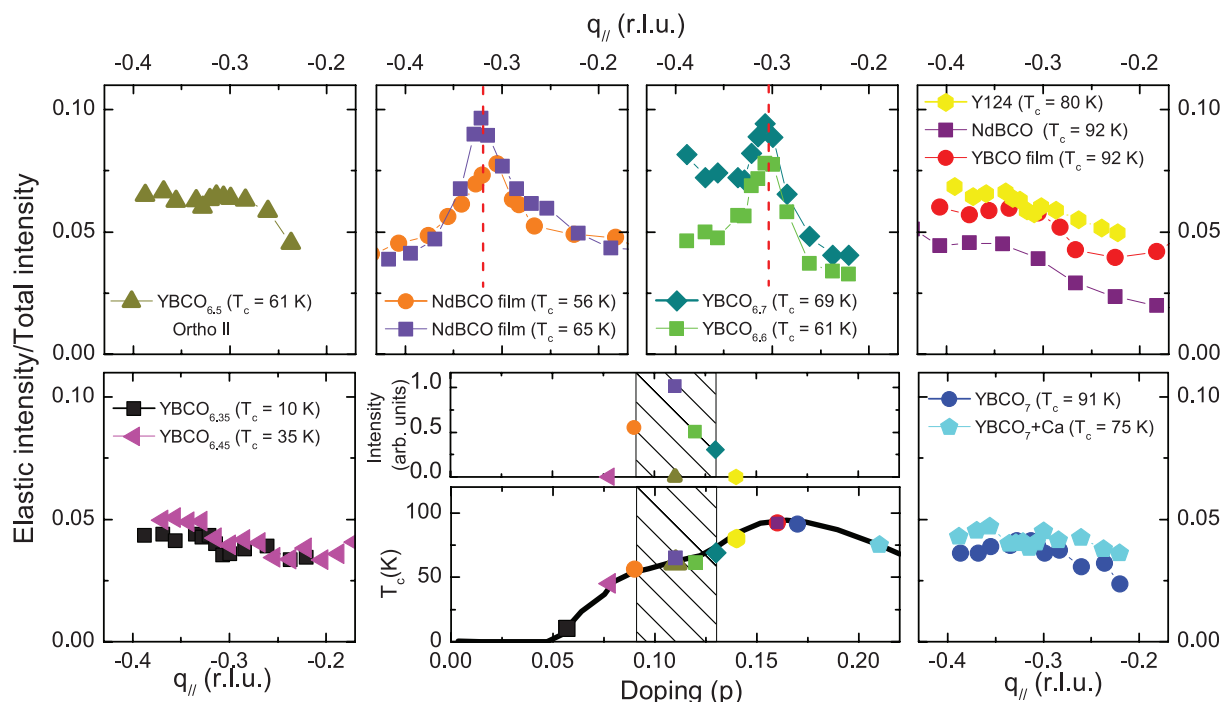


Fig. 3. Dependence of the CDW signal at 15 K on the hole doping level p . The CDW signal is present in several $\text{YBa}_2\text{Cu}_3\text{O}_{6+x}$ and $\text{Nd}_{1+x}\text{Ba}_{2-y}\text{Cu}_3\text{O}_7$ samples, but only for $0.09 \leq p \leq 0.13$. In this doping range (shaded in the

central panel), the T_c -versus- p relation exhibits a plateau. The CDW peak position does not change with p outside of the experimental error, but its intensity is maximum at $p \approx 0.11$.

tunneling spectroscopy (STS) on the surface of superconducting $\text{Bi}_2\text{Sr}_2\text{CuO}_{6+\delta}$ (2201) (30) and $\text{Bi}_2\text{Sr}_2\text{CaCu}_2\text{O}_{8+\delta}$ (2212) (31–33), but only limited information is available from STS on the temperature evolution of the CDW correlations in the 2201 and 2212 compounds, and the relation of the STS data to the superconducting properties in the bulk of these materials is obscured by electronic inhomogeneity.

The wave vector of the charge correlations revealed in our experiments is in good agreement with the nesting vector of the antibonding Fermi surface sheets predicted by density functional calculations for the 123 system (34). The nesting vector connects those segments on the 2D Fermi surface that develop the maximal gap amplitude in the d -wave superconducting state. The CDW is thus a natural consequence of a Fermi surface instability competing with superconductivity (35). The nearly critical nature of the charge fluctuations (Fig. 4) suggests a CDW ground state for underdoped 123 materials in magnetic fields sufficient to weaken or obliterate superconductivity, which may be responsible for the Fermi surface reconstruction evidenced by the quantum oscillation data (15). This scenario agrees qualitatively with a recent NMR study of $\text{YBa}_2\text{Cu}_3\text{O}_{6.5}$ in high magnetic fields, which has revealed signatures of a field-induced CDW (17), and with theoretical work on this issue (16, 36). In this precise case, the long range ortho-II oxygen order and/or the high-field conditions may favor the uniaxial commensurate charge modulation with period $4a$ inferred from these data. Further work is required to understand the interplay between the chain order and the in-plane charge modulation and to reconcile our results with the NMR data.

It is instructive to compare the charge correlations revealed by our RXS experiments to the p evolution of the spin correlations previously determined by magnetic neutron scattering. For $p \leq 0.08$, these experiments revealed incommensurate magnetic order with wave vectors $q_{\parallel} = (\frac{1}{2} + \delta, \frac{1}{2})$, where the incommensurability δ increases monotonically with p (27). Figure 3 shows that neither charge order nor low-energy incommensurate charge fluctuations are present in this doping range. For $p > 0.08$, the spin correlations remain centered at wave vectors similar to those at lower p , which bear no simple relation to the wave vector of the REXS peaks determined here (11–14). In this doping range, magnetic order disappears (27, 37, 38), and the magnetic excitation spectrum develops a large gap that increases smoothly with p , in stark contrast to the abrupt appearance and disappearance of the charge density correlations with increasing p .

These considerations imply that spin and charge order are decoupled in the 123 family, which is quite different from in the 214 family where they coexist microscopically in the striped state. The small or absent intensity difference between the signal intensities at $q_{\parallel} = (0.31, 0)$ and $(0, 0.31)$ (Fig. 2A) call into question the interpretation of various transport anomalies in the normal state of underdoped 123 compounds in terms of stripe fluctuations (8, 9), at least in the doping range where we observed the REXS peaks. Although further experiments are required to establish whether these peaks arise from an equal distribution of fluctuating domains of two uniaxial CDWs with mutually perpendicular propagation vectors or from a single CDW with biaxial charge modulation, the isotropic intensity distribution of the CDW signal shows that these fluctuations cannot account for the strongly anisotropic resistivity and Nernst effect in the normal state.

Despite these materials-specific variations, our data imply that long-range CDW correlations are a common feature of underdoped cuprates. Detailed microscopic calculations are required to assess their relation to the pseudogap phenomenon (1), the unusual $q = 0$ order (39), and the polar Kerr effect measurements (40) recently reported in some of these materials. The extensive q -, p -, and T -dependent data set we report here is an excellent basis for theoretical work on these issues.

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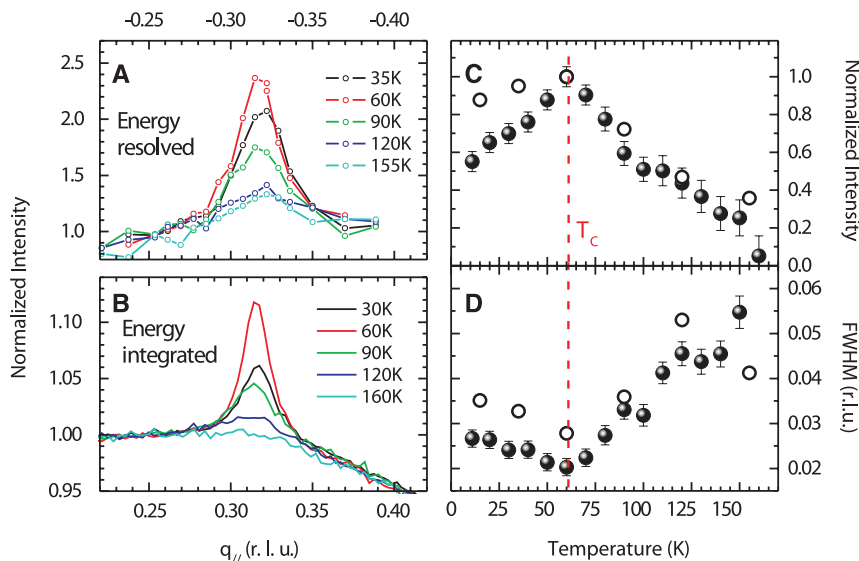


Fig. 4. Temperature dependence of the CDW signal in $\text{YBa}_2\text{Cu}_3\text{O}_{6.6}$. (A and B) Comparison of RXS scans at selected temperatures, as obtained with an energy-resolving instrument and with a conventional diffractometer for soft x-rays. (C and D) T -dependence of the CDW intensity and full width at half maximum (FWHM) derived from the energy-resolved (open circles) and the energy-integrated data (solid circles) for σ polarization.

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Supplementary Materials

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Long-Range Ordered Carbon Clusters: A Crystalline Material with Amorphous Building Blocks

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Solid-state materials can be categorized by their structures into crystalline (having periodic translation symmetry), amorphous (no periodic and orientational symmetry), and quasi-crystalline (having orientational but not periodic translation symmetry) phases. Hybridization of crystalline and amorphous structures at the atomic level has not been experimentally observed. We report the discovery of a long-range ordered material constructed from units of amorphous carbon clusters that was synthesized by compressing solvated fullerenes. Using x-ray diffraction, Raman spectroscopy, and quantum molecular dynamics simulation, we observed that, although carbon-60 cages were crushed and became amorphous, the solvent molecules remained intact, playing a crucial role in maintaining the long-range periodicity. Once formed, the high-pressure phase is quenchable back to ambient conditions and is ultra-incompressible, with the ability to indent diamond.

Carbon materials—such as graphene, graphite, diamond, fullerenes, and carbon nanotubes, as well as nanostructured and amorphous carbon—display a remarkable range of mechanical, electronic, and electrochemical properties that have led to many advanced applications (1–7). The structures of all of these materials are either ordered (crystalline) or disordered (amorphous). Polymeric fullerenes have been synthesized at different pressures and temperatures when pure C₆₀ or C₇₀ were used as starting materials (8–11). Under cold (ambient temperature) compression, the C₆₀ cages collapse, and the crys-

talline C₆₀ phase transforms into amorphous carbon above 30 GPa (12, 13). Under high-temperature compression, pure C₆₀ forms different polymerized structures, with the C₆₀ cages transforming into graphitic carbon or a mixture of *sp*² and *sp*³ amorphous phases. Some of these phases are also ultra-hard (8, 11, 14, 15).

Another type of starting material, solvated C₆₀, which is composed of C₆₀ molecules separated by solvent molecules, has received considerable attention (16–18). The solvated fullerenes are crystalline materials with high stability, tunable metrics, and functionality. The incorporation of guest molecules into the host C₆₀ lattice changes the crystal structure. The changes are also reflected in their vibrational properties and, consequently, the Raman and IR (infrared absorption) spectra (19). It was reported that the guest molecules can hinder the rotation of the C₆₀ molecules, leading to a decrease in their vibrational-rotational couplings (20, 21). Furthermore, the interactions between C₆₀ and the guest molecules reduce the icosahedral symmetry of the C₆₀ molecules, allowing electronic transitions that are forbidden in pristine C₆₀ and inducing a strong photoluminescent response (22).

C₆₀**m*-xylene, an important solvated C₆₀ with greatly enhanced photon luminescence (18, 22),

was selected for this investigation. It was studied up to 60 GPa by using a diamond anvil cell. X-ray diffraction (XRD), Raman spectroscopy, infrared absorption spectroscopy, and inelastic x-ray scattering (IXS, also called x-ray Raman spectroscopy) were used to analyze the crystal structure, lattice vibration, and bonding type of the material at high pressures. Independent quantum molecular dynamics (QMD) simulations were also carried out to understand and provide insight into the phase transformation of the material under high pressure.

We used XRD to reveal long-range structural order. Detailed information about the experiments is shown in the supplementary materials. Typical XRD patterns of the C₆₀**m*-xylene during compression are shown in Fig. 1. The XRD pattern at ambient pressure is well indexed as individual C₆₀ molecules occupying the lattice points of a hexagonal close-packed (hcp) structure (space group P6₃) with lattice constants *a* = 2.3761 nm and *c* = 1.0120 nm, in good agreement with literature values (18, 22, 23). During compression up to 60.1 GPa, the diffraction peaks gradually broaden, weaken, and shift to higher *Q* (reciprocal lattice vector). No drastic change was observed, indicating that the hcp periodicity of the molecules

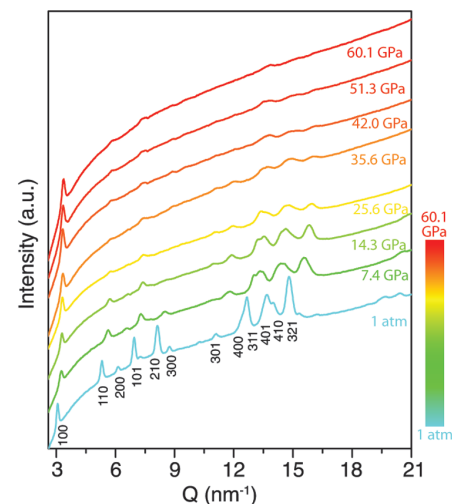


Fig. 1. XRD patterns of C₆₀**m*-xylene at different pressures. The numbers below the 1-atm XRD pattern indicate the indexing for the hcp structure. The pressures for the XRD patterns are given by the colored numbers at the right and in the bar.

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remains intact. This is in contrast to pure C_{60} , where the face-centered cubic periodicity of the C_{60} molecular units disappears when it amorphizes above 30 GPa (13).

We employed Raman spectroscopy to study the local structure of ordered amorphous carbon clusters (OACC) recovered from high pressures. The Raman spectrum of pristine C_{60}^* -*m*-xylene has 10 Raman-active modes ($2A_g$ and $8H_g$), as labeled in the Fig. 2A, arising from the intramolecular vibrations of C_{60} and some other weak peaks located from 300 to 550 cm^{-1} , which originate from the van der Waals interactions between C_{60} and the *m*-xylene molecules. Figure 2A shows the Raman spectra of the pristine sample and the samples decompressed from different pressures. As shown in the spectrum at 13 GPa, all of the vibrational bands that correspond to the caged structure remained, suggesting the preservation of the C_{60} cages. No peak shift was observed for the $A_g(2)$ mode, indicating that no pressure-induced polymerization occurred in the solvated C_{60} because the C_{60} cages are isolated from each other by the solvents (11). However, in the spectrum of the sample decompressed from 32 GPa, only a few strong bands from the C_{60} were observed, and all of the other bands disappeared. At the same time, a new band originating from the amorphous carbon appeared and became dominant at higher pressures. These observations suggest that C_{60} cages start to collapse at 32 GPa and completely transform into amorphous carbon clusters at higher pressures (12, 13).

The two broad bands preserved in the spectrum of the sample decompressed from 32 GPa contain three Raman peaks correspond to the pentagon shear [$H_g(7)$], pentagon pinch [$A_g(2)$], and hexagon shear [$H_g(8)$] modes, respectively (8, 9, 11). The vibrational modes related to the breathing modes (at lower wave numbers) of the cage disappear. These changes suggest that C_{60} cages

start to break into fragments and lose the cage symmetry, but the pentagon and hexagon rings are still preserved in the fragments, as evidenced from the Raman peaks observed in the recovered sample. At higher compression, the fragments are increasingly broken up, and thus we only observe a very broad peak from amorphous carbon.

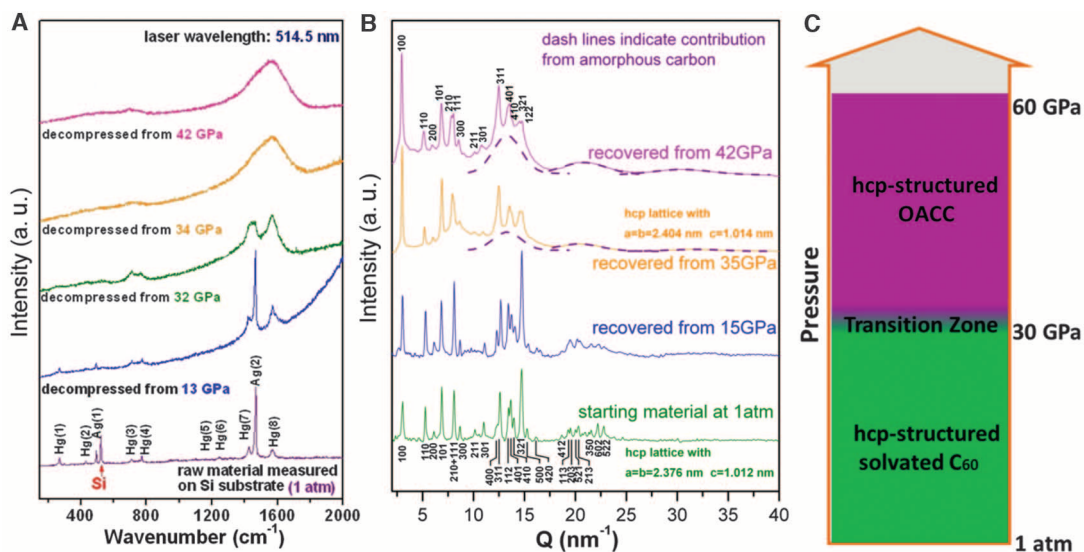
We further studied the bonding change of the sample during the phase transformation by using IXS. The experimental details and spectra (fig. S1) are included in the supplementary materials. As labeled in the figure, both π^* and σ^* peaks, which correspond to sp^2 and sp^3 bonds, were observed in the spectra at relatively low pressures, and no obvious change in peak intensities was observed below 26 GPa (6). At higher pressures above 36 GPa, the π^* peak became too weak to be observed, suggesting the complete bonding transition from sp^2 to sp^3 caused by the collapse of C_{60} cages as observed by the Raman spectroscopy. The IXS spectrum of OACC recovered from 42 GPa shows a π^* peak that is much weaker in intensity compared with lower compressions, suggesting that a portion of sp^3 bonding transforms back to sp^2 upon decompression while the rest is preserved to the ambient pressure. This implies that OACC has mixed bonding with both sp^2 and sp^3 .

The ability to preserve the high-pressure structure back to ambient conditions is important for future practical applications. Figure 2B shows XRD patterns of a pristine sample and the samples decompressed from different pressures. We found that at pressures below ~ 30 GPa, the C_{60} cages survive and are recovered upon decompression. As the applied pressure is increased, the C_{60} cages start to be irreversibly crushed, and this disorder is preserved back at ambient pressure. The C_{60} cages completely collapse and transform into amorphous clusters at pressures above 32 GPa, but the overall hcp lattice is preserved, forming

OACC (Fig. 2C). The XRD for the decompressed OACC retains long-range periodicity similar to the pristine material. As shown in the figure, OACC recovered from 35 GPa has a hcp structure (space group $P6_3$) with $a = 2.404\text{ nm}$ and $c = 1.014\text{ nm}$. The lattice is slightly larger than the pristine structure due to the collapse of the C_{60} cages. In addition, there are several broad bands (indicated by the dashed lines) that coexist with the sharp diffraction peaks in the spectra of the decompressed samples. Because the background has been carefully subtracted during the measurements, the broad bands represent the contribution from the amorphous carbon clusters, which is consistent with the local disorder observed in the Raman studies. The center position of the first band is $\sim 4.4\text{ \AA}$, which is different from glassy carbon and other amorphous carbons that possess some short-range order. Although the physical meaning of the first sharp peak of an XRD pattern from an amorphous material is not completely understood, the difference still suggests that the amorphous carbon clusters in OACC have different local structure with glassy carbon and other amorphous carbons due to their extremely small size and different local environments.

Previous experimental and simulation results suggest that the solvent molecules are placed along the three-fold axis of the lattice (22, 23). Therefore, the C_{60} cages are isolated from each other by the solvent. Although the C_{60} cages collapse at high pressures, the carbon clusters likely stay on the lattice sites and maintain the periodic structure due to the presence of the solvent molecules (fig. S2). This finding suggests that the solvent plays a crucial role in maintaining the long-range periodicity in OACC. The solvent in the solvated fullerenes can be easily removed by a heat treatment (18). Figure S3 in the supplementary materials shows the XRD pattern of the decompressed material after a mild heat treatment

Fig. 2. Raman spectra and XRD patterns of samples decompressed from different maximum pressures and the phase diagram at room temperature. **(A)** The Raman spectra were gathered using a 514.5-nm excitation laser line. In the spectra of the samples quenched from pressures above 32 GPa, all the bands belonging to C_{60} disappeared and a new band originating from the amorphous carbon appeared and became dominant. **(B)** The backgrounds were carefully subtracted during the measurement of the XRD patterns. There are several broad bands (indicated by the dashed lines) that represent the contributions from the amorphous carbon clusters and coexist with the sharp diffraction peaks from the hcp lattice. **(C)** The phase diagram shows that below about 30 GPa the C_{60} cages survive and can be recovered upon decompression. At higher pressures, the C_{60} cages start to irreversibly break and transform into hcp-structured OACC.



at 107°C for 8 hours. The material loses its long-range periodicity and transforms into an amorphous structure after the solvent is evaporated, further confirming the critical role of the solvent in maintaining long-range periodicity. Furthermore, the structure of solvated fullerenes (e.g., C₆₀ and C₇₀) is solvent dependent. An extensive

class of crystalline-solvated fullerenes can be obtained by changing the solvent species and the ratio of fullerenes to solvent (22). This result suggests that one may be able to synthesize a series of carbon materials with this unique structure, but with different packing symmetry and carbon cluster size, by selecting different fullerene cages.

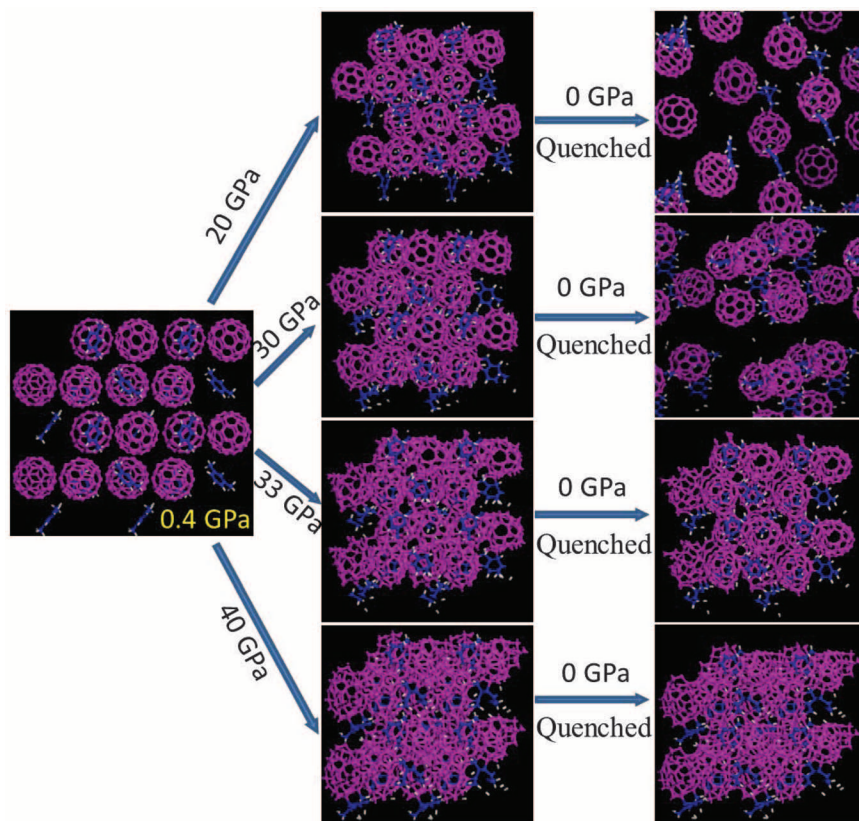


Fig. 3. Simulated structures of the material under different compression and decompression conditions. Far left, the pristine structure at 0.4 GPa. The deformation of the C₆₀ cages is elastic below ~30 GPa, and the deformed cages return to their initial shape as they are decompressed back to ambient pressure. Above 30 GPa, many carbon-carbon bonds start to break, and the cages can no longer return to C₆₀ upon decompression. OACC maintains long-range periodicity and can be preserved under ambient pressure conditions.

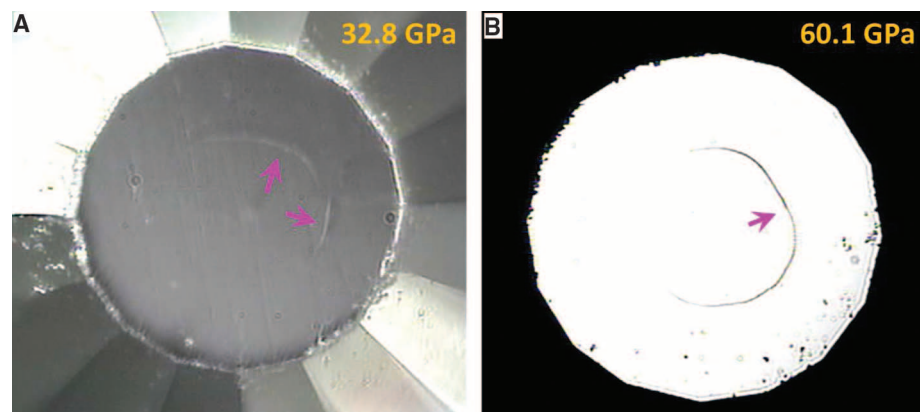


Fig. 4. Optical photomicrographs of the ring cracks in the diamond anvils generated after formation of OACC and then release of the pressure. The diamonds were carefully cleaned using sandpaper. The maximum pressures were 32.8 (A) and 60.1 GPa (B). As marked by the arrows, the ring cracks follow the original boundary of the samples in the gaskets.

The experiments show the phase transformation and evidence for the existence of OACC. To demonstrate that the experimentally observed OACC is a generic phenomenon, and to provide molecular-level insight into how this new material forms at high pressure, we performed independent QMD simulations using a similar close-packing (face-centered cubic) system as a test model system. Detailed information about the calculation is shown in the supplementary materials. Figure 3 shows the structures of the materials at different pressures and upon decompression. Below ~30 GPa, the deformation is elastic. The deformed cages return to their initial shapes as they are decompressed back to ambient pressure. However, above 30 GPa, the deformation becomes so large that a phase transition is triggered, and C₆₀ molecules start to collapse by breaking the *sp*² hybrid C-C double bonds and forming new *sp*³ hybrid C-C bonds. The spherical-shaped C₆₀ molecules are also changed to the ellipsoidal structure. As a result, the cages can no longer return to their initial structure upon decompression. At even higher pressures (>35 GPa), the C₆₀ cages completely collapse and transform into highly disordered carbon clusters forming OACC; OACC maintains the long-range periodicity due to the presence of the solvent molecules and can be preserved back to ambient pressure conditions. The pressure-induced phase transition seen in QMD simulations at about 32 to 33 GPa is consistent with that observed in the experiments, thereby offering additional insight into the local structure of the new material, as well as physical and chemical mechanisms of this phase transition.

Finally, it is important to note that OACC created ring crack indentations on the diamond anvils. These indentations follow the original boundary of the sample in the gasket, indicating the exceptional hardness of the new phase (4, 5). Two optical pictures of the ring cracks generated after releasing from pressures of 32.8 and 60.1 GPa are shown in Fig. 4. From previous studies, ring cracks have only been observed when a diamond anvil is indented by another superhard material, such as an opposing beveled diamond anvil. The observation of ring cracks indicates that OACC has ultra-incompressibility comparable to diamond. The shift of the strongest XRD peak of the high-pressure phase as a function of pressure was also analyzed (fig. S11). Comparison with diamond also suggests that OACC is as incompressible as diamond.

In conclusion, we synthesized a carbon material, from units of amorphous carbon clusters and solvent molecules organized on a crystalline lattice, with the incompressibility comparable to diamond. OACC has long-range periodicity but is locally disordered, with the solvent molecules playing a crucial role in maintaining the long-range periodicity. It can also be preserved back to ambient conditions. Simulations provide additional insight into the formation mechanism that are consistent with the experimental results.

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Supplementary Materials

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Camouflage and Display for Soft Machines

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Synthetic systems cannot easily mimic the color-changing abilities of animals such as cephalopods. Soft machines—machines fabricated from soft polymers and flexible reinforcing sheets—are rapidly increasing in functionality. This manuscript describes simple microfluidic networks that can change the color, contrast, pattern, apparent shape, luminescence, and surface temperature of soft machines for camouflage and display. The color of these microfluidic networks can be changed simultaneously in the visible and infrared—a capability that organisms do not have. These strategies begin to imitate the functions, although not the anatomies, of color-changing animals.

Cephalopods (such as squid and cuttlefish) have amazing control over their appearance (color, contrast, pattern, and shape) (1, 2). These animals use dynamic body patterns for disguise, for protection, and for warning. Other animals (such as chameleons and many insects) can also actively change their coloration for camouflage or display (3, 4). Still others (such as jellyfish and fireflies) use bioluminescence to communicate (5). The color-changing capabilities of these animals have not been replicated by using soft synthetic systems, but such systems could enhance the function of certain machines (such as robots or prosthetics).

This paper describes our initial approaches to change the color, contrast, pattern, apparent shape, luminescence, and infrared (IR) emission (that is, surface temperature) of soft machines fabricated from elastomers and flexible reinforcing sheets (6–8) by changing the color and pattern of microfluidic networks. These systems are first steps toward imitating the functions, al-

though not the anatomies, of cephalopods (9, 10) and other color-changing animals (4). These animals typically change color using specialized cells, such as chromatophores or iridophores (4, 9, 10), not simple microchannels. The near-perfect matching of environments used by color-changing organisms with highly developed nervous systems is not required for camouflage to be effective.

Nature offers countless examples of camouflage and display (3, 11, 12). Although specific demonstrations of camouflage vary among species, the strategies used have common themes: background matching, disruptive coloration, and disguise (3, 11, 12). In background matching, animals use colors and patterns similar to their habitats (3, 11–13), such as counter/obliterative shading (for example, rabbits with white underbellies and brown backs) (11, 12). Disruptive coloration breaks up the silhouette of an animal using contrasting patterns that do not follow its shape (3). Disguise is a strategy in which an organism adopts the shape and color of objects or animals in the environment (such as stick insects and phasmida) (3). Display strategies are used for communication, mating, and hunting and can be just as critical for survival as camouflage (3). Bioluminescence is commonly used for display

in low- or no-light conditions (5, 14); when light is strong, animals sometimes advertise themselves by combining vibrant contrasting colors with distinct shapes (15–17). For example, jellyfish use bioluminescence to warn would-be predators (5), and peacocks use bright plumage to attract mates (18).

Although most strategies of camouflage and display (3, 12) emphasize the visible spectrum that most animals (especially mammals) can see (19–21), there are animals that can see (or otherwise sense) ultraviolet (UV) or IR light and use these spectral regions for signaling or hunting. For example, most birds, many arthropods, and some fish have UV vision (22), and many snakes, such as pit vipers, can sense IR light using specialized organs (23). Semiconductor technology has expanded our ability to see into the IR (24), and we explored techniques for camouflage or display in the IR.

Our system combines microfluidics, pattern, and color to provide both camouflage/display and movement of the soft machines. Camouflage or display results from pumping colored or temperature-controlled fluids through a network of microfluidic channels; mechanical actuation results from pneumatic pressurization and inflation of an independent network of microchannels (pneu-nets) embedded in highly extensible elastomers (6, 7). The microfluidic networks used for camouflage/display are contained in thin silicone sheets [Ecoflex (Smooth-On, Easton, PA), 1 to 2 mm thick] referred to as color layers (Fig. 1 and fig. S1). Although there are technologies, such as electrowetting (25) and electrofluidics (26), that use microfluidics to tune color, they rely on electric fields to move fluid and are not immediately compatible with our compressed-air power source.

The color layers are easily fabricated (27), can cover large areas, and can transport a variety of fluids (both liquids and gases) using similar controls (28). The liquids can be colored with dyes or pigments and heated/cooled to change the color of the microfluidic networks in the visible and IR

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spectrum; the possible combinations of colorants at different temperatures provide greater flexibility in spectral tailoring than can be achieved by using other technologies: Thermoelectrics can change the IR signature, and electronic displays can change the visible color, but neither technology has control over both IR and visible coloration. Further, once filled, the color layers require no power, have low requirements for volume of fluid ($\sim 30 \mu\text{L}/\text{cm}^2$ of surface), and are lightweight ($130 \text{ mg}/\text{cm}^2$ of surface). We used closely packed microfluidic channels (Fig. 1) or combined microfluidic channels with wider (millimeters to centimeters) channels (fig. S1D) to create

features that are indistinguishable from continuous color in the far field. Ecoflex, unlike the materials used in commercially available flexible electronic displays, has a Young's modulus that is compatible with the flexible bodies and motions of soft machines and is also mechanically robust and inexpensive. The translucency of Ecoflex also helped the machines blend in with their surroundings.

We used a soft robotic quadrupedal “walker” (7) to demonstrate that the color layers are light and flexible enough to be compatible with a mobile and stretchy soft machine. We took advantage of the regular and symmetrical shape of the

quadruped to illustrate camouflage strategies that can break up recognizable silhouettes.

We attached the color layers to the top of soft quadrupeds (Fig. 1, D to E), which were fabricated as described previously (6, 7). Using a pumping rate of $2.25 \text{ mL}/\text{min}$ and neglecting the time necessary to fill the tether, a change in color required 30 s (Fig. 1D) [color-change was also monitored with reflectance spectroscopy (fig. S6)]. The weight and flexibility of the color layer did not substantially slow the robot's locomotion (the velocity was 0.6 times the velocity of the same robot without the color layer) (Fig. 1E) (7).

Illustrated in Fig. 2 are the operation of camouflage in a rock bed (movie S1) and a leaf-covered concrete slab using two different color layers (Fig. 1 and fig. S1D) filled with two different sets of colored solutions. In all demonstrations, the colored solutions were prepared manually by the operator. Both designs created large, disjointed patches of contrasting brightness similar to the environment but not to the shape of the robot; this disjuncture in shape [disruptive coloration (3)] helped conceal the robot. Simple image analysis performed on the camouflaged and uncamouflaged robot (Figs. 2B and 3 and figs. S4 and S5) demonstrated that the brightness of the camouflaged robot was significantly closer to the brightness (within the deviation defined by root mean square contrast) (fig. S4) of the environment than that of the uncamouflaged robot (27, 29). Additionally, we found the similarity in pattern between the background and the camouflaged robot to be favorable (fig. S5). This brightness/contrast resemblance and similarity in pattern was important to this demonstration.

We also investigated the camouflage of soft robots [using background matching (3, 11–13)] in a patterned environment similar to a tiled floor (Fig. 2, E to F, and fig. S2) by creating a color layer with periodic microfluidic channels filled with colors matched to that environment. Although not perfectly camouflaged, the colored robots are less obvious and demonstrate the potential of this system. Dynamic camouflage would be useful for applications in which soft machines must do their job without standing out (for example, soft robots performing maintenance operations).

Patterns and contrasting coloration schemes that make the soft machine stand out against a background (display), although less technically demanding than camouflage, are just as important and useful (an example would be to aid location and recovery of soft machines from poorly lit or cluttered environments). We changed the camouflage/display modes of the color layers simply by changing the fluid within them (Fig. 1C, bottom). This ability of a single color layer to switch between modes of coloration is an advantage. We further illustrate display by filling dense, shape-matching color layers (fig. S1B) with fluorescent (Fig. 2, G to H, and movie S2), black and white (Fig. 2J), or chemiluminescent (Fig. 2I and movie S3) solutions that revealed the location of the robots in light or dark.

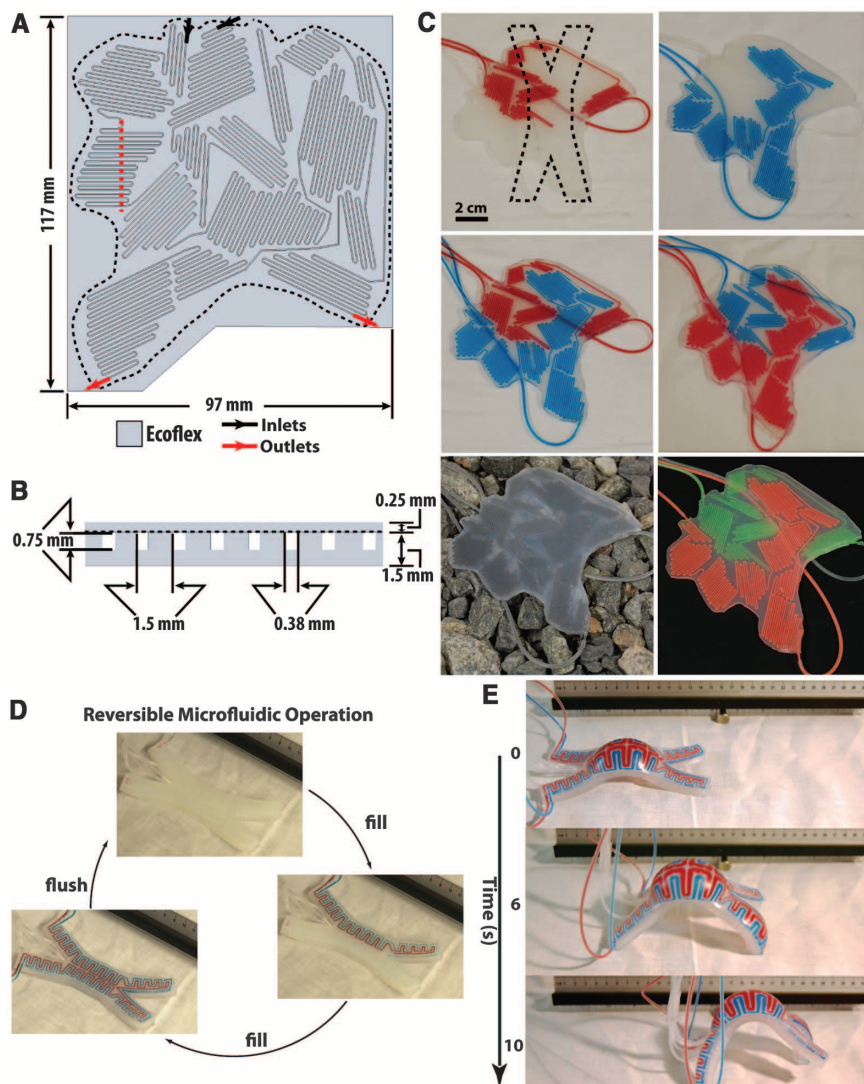


Fig. 1. Design and operation of a color layer. (A) Schematic of a disruptive color layer comprising two channels. The dotted black line indicates the final shape. (B) Cross-sectional schematic of the region indicated by the dotted red line in (A). (C) Various patterns of coloration generated by filling (or not filling) the channels of a completed color layer with solutions of dye (top four images) and pigment dispersions (bottom two images). The dotted line (top left) shows the outline of the quadrupedal soft robot. (D) Reversible coloration. This color layer design shows the operation of the system and is not intended to camouflage/display the machine. Colored aqueous solutions are pumped by using a syringe. The pumping rate was $2.25 \text{ mL}/\text{min}$ (channel volume = 0.75 mL). (E) Despite the added weight of a filled color layer, a soft quadruped moves at $\sim 40 \text{ m/h}$ (0.6 times the velocity of the same device without the color layer). The major ruler divisions are 1 cm.

We also demonstrated camouflage and display in the IR spectrum (Fig. 4 and movie S4) using warm (70°C) or cool (2°C) colored solutions. Pumping these temperature-controlled solutions through the color layer created contrasting IR color patterns that make the system stand out against its thermal environment. This strat-

egy makes it possible simultaneously to change the visible and IR colors. As an illustration, we camouflaged a color layer within the visible spectrum while simultaneously displaying it in the IR spectrum (Fig. 4, F to G). At a pumping rate of 2.25 mL/min, we achieved IR color change in 80 s (movie S4). The rate of IR color

change was limited by the thermal conductivity of Ecoflex [0.16 Wm⁻¹K⁻¹ (27)] and not the pumping rate.

Simple systems can approach some of the functionality of dynamic coloration that many animals use to control their appearance (4). Microfluidic networks that make up a color layer that is

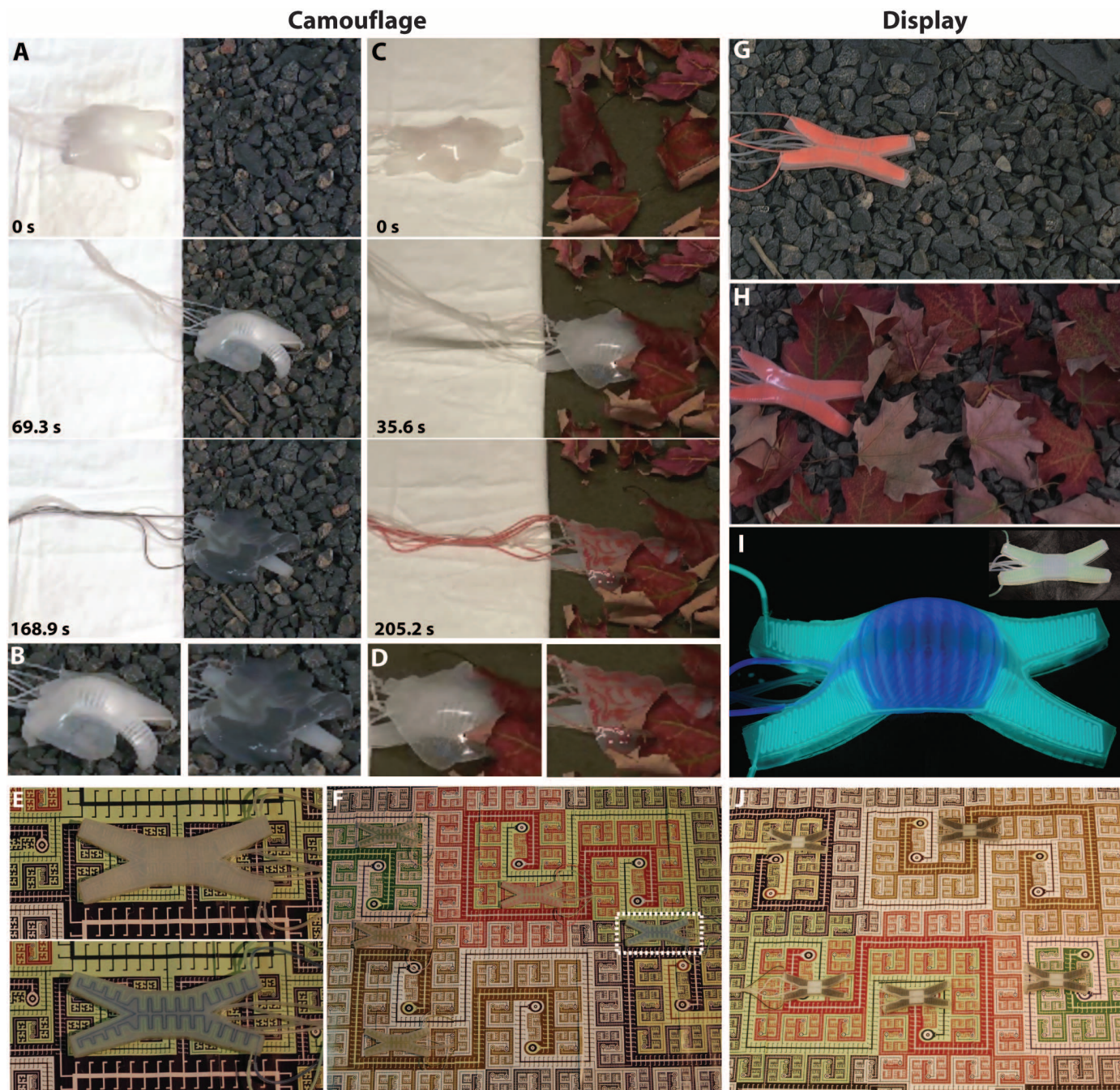


Fig. 2. Camouflage and display coloration. (A) Sequential images of a soft robot walking onto a rock bed, where it is camouflaged by pumping colored pigment dispersions through the microfluidic channels in the color layer. The number at the bottom left corner of each frame is the time (seconds). The tether is intentionally left visible to contrast the colors used against a white background. (B) Close-up images of the robot uncamouflaged (left) and camouflaged (right). (C and D) Images of a soft robot walking onto a leaf-covered concrete slab, where it is camouflaged. (E) Close-up image of an uncamouflaged (top) soft

robot in an artificial, man-made environment and the same soft robot camouflaged (bottom). (F) The robot from (E) in five different regions adopting coloration for background matching. One position of the robot from (E) is marked with a dashed box; all five are shown in fig. S3. (G) Fluorescent orange robot displayed on a rock bed. (H) Robot from (G) displayed on a leaf-covered rock bed. (I) Robot glowing in the dark using chemiluminescence. (Inset) The same robot photographed in the light. (J) Robots displayed in contrasting black and white on the pattern from (F). All quadrupeds were 13 cm long (Fig. 1).

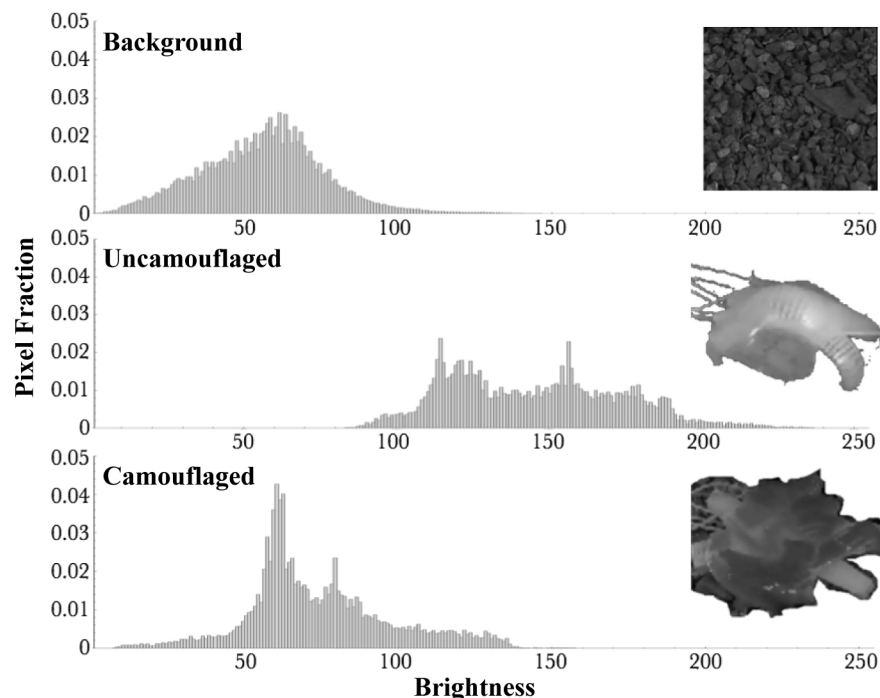


Fig. 3. Stacked histograms showing the distribution of gray-scale intensities (brightness) generated by image analysis of a rocky environment (background), an isolated uncamouflaged soft robot, and an isolated camouflaged soft robot. (Insets) The analyzed images.

independent of other functions can effectively—albeit crudely when compared with animals—camouflage or display soft machines by altering their apparent shape, color, contrast, luminescence, temperature, and pattern. The ability of color layers simultaneously to change color in the visible and the IR is a capability not used by organisms and not easily replicated by using other technologies: Animals are limited in their ability to control their temperature; soft machines fabricated in silicone elastomers are not.

These devices confirm that microfluidic channels can serve multiple functions in soft machines: actuation, camouflage, display, fluid transport, and temperature regulation. Combining different functional microfluidic systems simplifies the design and increases the functionality of soft machines. Complex/specialized microfluidic networks can take the place of these relatively simple color layers to bring more capabilities (such as reagent handling and storage) to soft machines.

We believe that the simple design, fabrication, and operation of these color-changing soft machines make these systems accessible and potentially useful to many different scientific fields. Biologists studying camouflage/display could use specially designed soft machines to observe how dynamic color, temperature, and pattern influence

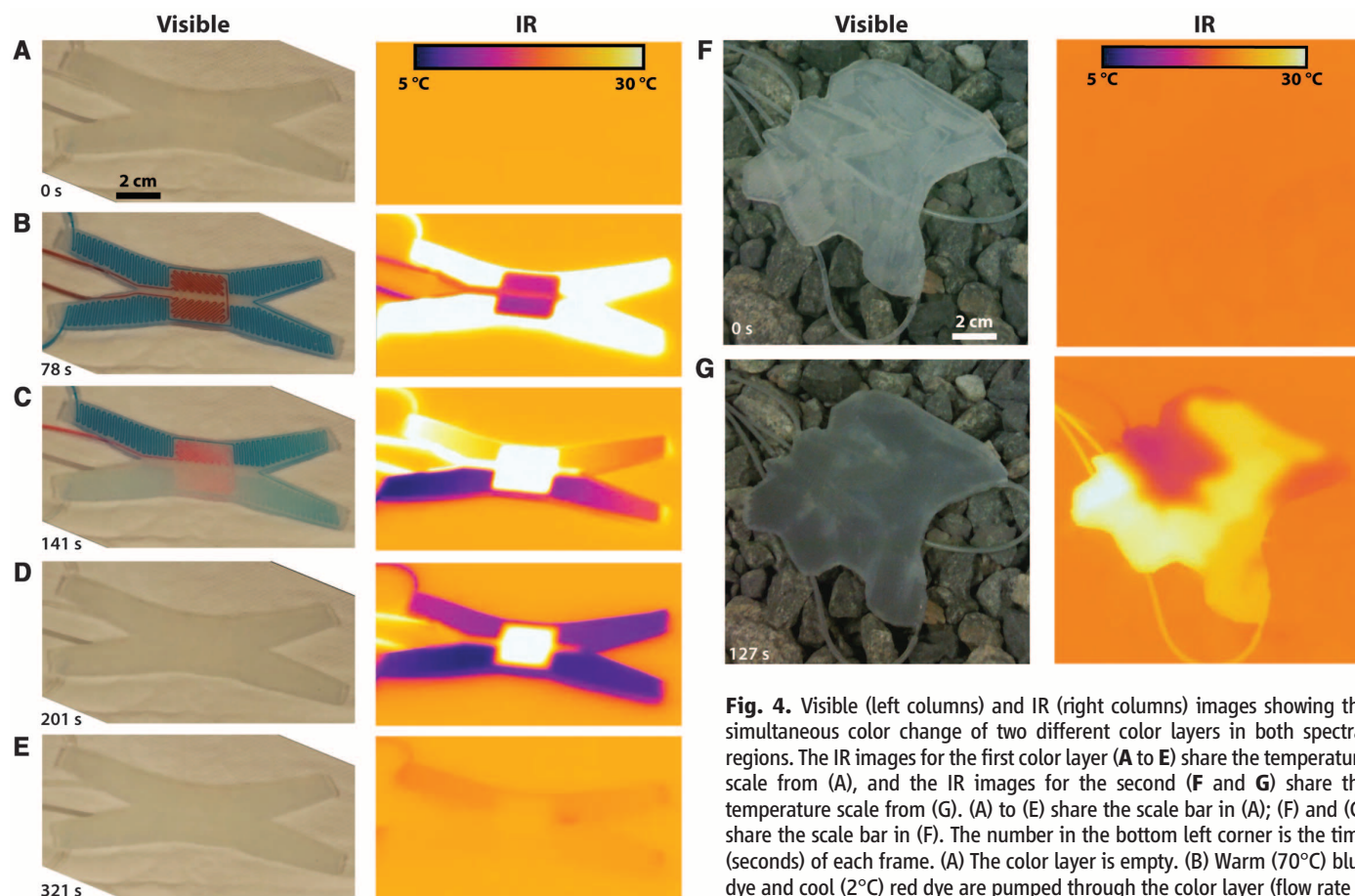


Fig. 4. Visible (left columns) and IR (right columns) images showing the simultaneous color change of two different color layers in both spectral regions. The IR images for the first color layer (A to E) share the temperature scale from (A), and the IR images for the second (F and G) share the temperature scale from (G). (A) to (E) share the scale bar in (A); (F) and (G) share the scale bar in (F). The number in the bottom left corner is the time (seconds) of each frame. (A) The color layer is empty. (B) Warm (70°C) blue dye and cool (2°C) red dye are pumped through the color layer (flow rate = 2.25 mL/min). [(C) to (D)] The dyes are exchanged with 2°C water and 70°C water. (E) Room temperature (22°C) water is pumped into both channels. (F) An unfilled color layer is seen in the visible but camouflaged in the IR. (G) The color layer from (F) is camouflaged in the visible by using two shades of gray pigment dispersions. One dispersion is at 70°C, and the other is at 2°C.

water. (E) Room temperature (22°C) water is pumped into both channels. (F) An unfilled color layer is seen in the visible but camouflaged in the IR. (G) The color layer from (F) is camouflaged in the visible by using two shades of gray pigment dispersions. One dispersion is at 70°C, and the other is at 2°C.

animal behavior (such as predator/prey relationships) (13, 30). In anatomy, our devices could simulate fluid vessels and muscle motion for realistic modeling or training. Although we have focused on soft robots, our display systems can also interface with hard robots and present new opportunities for modifying the appearance of these devices. These applications are approachable by using tethered microfluidic systems such as those demonstrated here, but some applications will demand more technically advanced autonomous systems. The challenges associated with autonomy will be addressed initially by using larger-bodied machines or robots that can carry power sources, pumps, electronics, and fluids.

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Supplementary Materials

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Movies S1 to S4

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Mixed-Phase Oxide Catalyst Based on Mn-Mullite (Sm, Gd)Mn₂O₅ for NO Oxidation in Diesel Exhaust

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Oxidation of nitric oxide (NO) for subsequent efficient reduction in selective catalytic reduction or lean NO_x trap devices continues to be a challenge in diesel engines because of the low efficiency and high cost of the currently used platinum (Pt)-based catalysts. We show that mixed-phase oxide materials based on Mn-mullite (Sm, Gd)Mn₂O₅ are an efficient substitute for the current commercial Pt-based catalysts. Under laboratory-simulated diesel exhaust conditions, this mixed-phase oxide material was superior to Pt in terms of cost, thermal durability, and catalytic activity for NO oxidation. This oxide material is active at temperatures as low as 120°C with conversion maxima of ~45% higher than that achieved with Pt. Density functional theory and diffuse reflectance infrared Fourier transform spectroscopy provide insights into the NO-to-NO₂ reaction mechanism on catalytically active Mn-Mn sites via the intermediate nitrate species.

Diesel engines are attractive because of their higher fuel efficiency than gasoline engines (1). However, their lean exhaust with high oxygen (O₂) content requires specialized devices to reduce the engine-generated nitrogen oxide (NO_x) pollutants, mostly nitric oxide (NO), to environmentally benign nitrogen (N₂). One popular strategy is the use of selective catalytic reduction (SCR) of NO_x with ammonia (NH₃) through the reaction: 2NH₃ + NO + NO₂ → 2N₂ + 3H₂O. The rate of this reaction is maximized (fast SCR) when the NO:NO₂ ratio approaches unity (2, 3). A lean NO_x trap (LNT) adsorbs NO_x under lean conditions and reduces NO_x to N₂ under fuel-rich regeneration cycles. NO oxidation products such as nitrogen dioxide

(NO₂) have a higher trapping efficiency on LNT materials (4). In addition to the NO_x-based pollutants, diesel engines also generate black soot with polynuclear aromatic content. One process to remove carbon soot is a continuous regenerable trap (CRT), which uses NO₂ as an oxidant (2NO₂ + C → CO₂ + 2NO, NO₂ + C → CO + NO). Oxidation of engine-generated NO to NO₂ is critical in diesel engine emission control for reducing NO_x and carbon soot from the tail-pipe exhaust.

To date, no thermally stable material has matched platinum's catalytic performance under diesel exhaust temperatures, despite a substantial amount of work that has been undertaken in search for catalysts based on metal oxides (5, 6). Here, we report a class of hydrothermally stable,

mixed-phase oxides based on Mn-mullite, (Sm, Gd)Mn₂O₅. This catalyst demonstrates activity at temperatures as low as 120°C and has a ~64% increase in NO oxidation catalytic performance over 2% Pt on γ-Al₂O₃ at 300°C. The catalytic behavior is mainly linked to the Mn-Mn dimers on the mullite surface based on characterization methods and quantum-mechanical simulations.

Mixed-phase oxide catalysts were prepared by a coprecipitation method (7). Transition metal, alkaline earth, and rare earth precursors were dissolved in water with a non-ionic surfactant. The pH was adjusted to between 9 and 10 with tetramethylammonium hydroxide, followed by the addition of oxalic acid and hydrogen peroxide. The precipitate was filtered, dried, calcined, and hydrothermally aged (materials and methods S-I).

To evaluate catalytic performance, we exposed the aged sample Mn₇CeSmSrO_{14.83}, labeled MnCe-7:1, to the reactant gas mixture (450 parts per million NO and 10% O₂) while ramping the temperature up to 350°C to test for NO conversion (materials and methods S-II).

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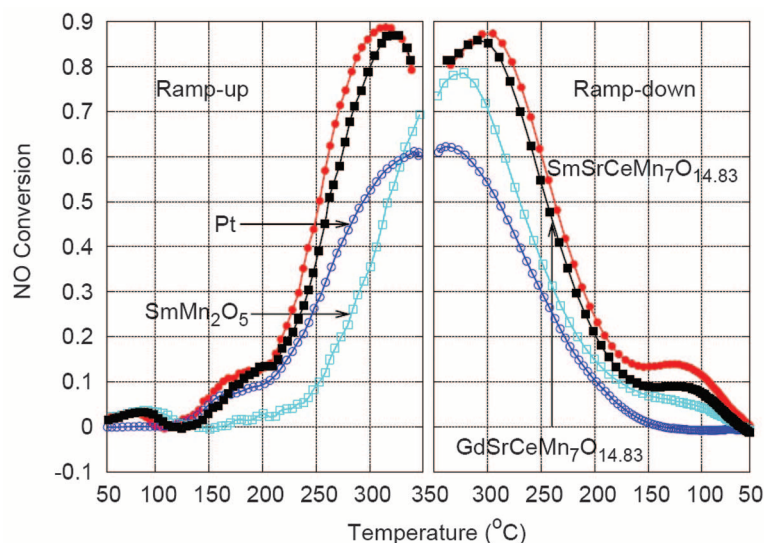


Fig. 1. NO conversion versus ramp-up and ramp-down temperatures for MnCe-7:1 (●), SmMn_2O_5 (□), $\text{GdSrCeMn}_7\text{O}_{14.83}$ (■), and Pt (○).

Figure 1 demonstrates that aged MnCe-7:1 offers improved NO oxidation over the aged 2% Pt on $\gamma\text{-Al}_2\text{O}_3$ catalyst, and pure SmMn_2O_5 displays reasonable activity during the ramp-down cycle. Furthermore, MnCe-7:1 is active at low temperature (120°C), with a higher NO conversion maximum of $\sim 45\%$ over Pt on the ramp-up cycle. BET measurements (8) revealed that the sample maintained a surface area of $\sim 26\text{ m}^2/\text{g}$ before and after hydrothermal aging.

X-ray diffraction (XRD) was used to characterize the phases, composition, and average crystallite size of MnCe-7:1 (materials and methods S-III). The XRD spectrum in Fig. 2A shows three crystalline phases: mullite [$\text{Sm}(\text{Ce}, \text{Sr})\text{Mn}_2\text{O}_5$], spinel (Mn_3O_4), and fluorite (CeO_2), with corresponding concentrations of 65.9, 8.2, and 25.9% by weight, respectively. To explore the microstructure and the particle distribution in MnCe-7:1, scanning electron microscopy (SEM) and high-resolution transmission electron microscopy (HRTEM) were used (materials and methods S-IV). In Fig. 2B, SEM results display average

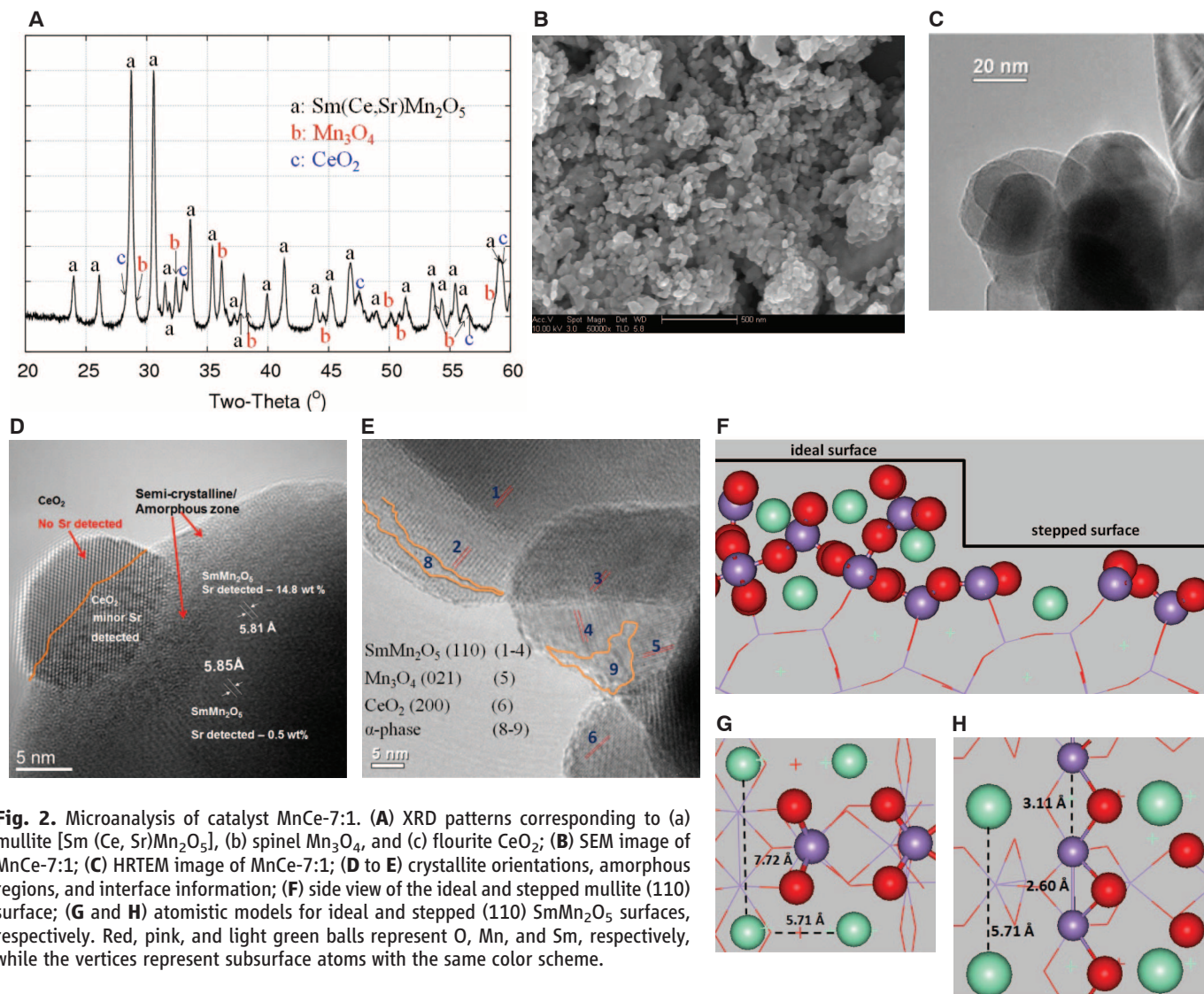


Fig. 2. Microanalysis of catalyst MnCe-7:1. (A) XRD patterns corresponding to (a) mullite [$\text{Sm}(\text{Ce}, \text{Sr})\text{Mn}_2\text{O}_5$], (b) spinel Mn_3O_4 , and (c) fluorite CeO_2 ; (B) SEM image of MnCe-7:1; (C) HRTEM image of MnCe-7:1; (D to E) crystallite orientations, amorphous regions, and interface information; (F) side view of the ideal and stepped mullite (110) surface; (G and H) atomistic models for ideal and stepped (110) SmMn_2O_5 surfaces, respectively. Red, pink, and light green balls represent O, Mn, and Sm, respectively, while the vertices represent subsurface atoms with the same color scheme.

particle sizes of ~ 50 nm, which is consistent with the XRD line-broadening results (SmMn_2O_5 , 47.4 nm; Mn_3O_4 , 34.9 nm; CeO_2 , 19.0 nm). These particles form interfaces as displayed in Fig. 2, C to E. HRTEM images (Fig. 2, D and E) reveal the crystallite orientations of SmMn_2O_5 (110), Mn_3O_4 (021), and CeO_2 (200) and show that CeO_2 forms an interface with the amorphous zone adjacent to the mullite. Within the amorphous region (Fig. 2D), a high concentration [14.8 weight percent (wt %)] of Sr is detected by energy-dispersive x-ray spectroscopy (EDS). Comparatively, a lower Sr concentration, ~ 0.7 wt %, was identified in the SmMn_2O_5 mullite phase.

To understand the NO oxidation reaction, we performed diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) on MnCe-7:1 (materials and methods S-V). Figure 3 depicts the time evolution of NO_x species at 200°C in NO and $\text{NO} + \text{O}_2$ gas environments. Density functional theory (DFT) calculations (materials and methods S-VI) were conducted to determine the wavenumbers of nitrosyl (NO), nitrite (NO_2), and nitrate (NO_3) on the relevant (110) mullite surface (supplementary text S-VIII). Wavenumbers of NO_x species on manganese oxides have been reported (9). Gas-phase NO exhibits N-O stretching frequencies at 1911 and 1846 cm^{-1} . Bands located at 1630 and 1600 cm^{-1} likely correspond to bidentate Mn-nitrate and monodentate Mn-nitrite on the mullite surface, respectively. Also, the NO_2 (g) molecular stretching mode (10) is 1628 to 1654 cm^{-1} , which overlaps with NO_x species on the surface. NO_x species on Sm sites are found at 1553 cm^{-1} (nitrate) and 1309 cm^{-1} (nitrite). Ce-correlated bands, centered at 1400 cm^{-1} (11), are not found. As shown in Fig. 3, with only NO flowing through the sample, bands positioned at 1600 and 1630 cm^{-1} (Mn-nitrite) diminish over time, indicating the consumption of surface oxygen on the mullite. When O_2 is introduced with the existing NO feed gas, these two specific bands are restored. This displays an equilibrium between Mn- NO_x formation and conversion into gas-phase NO_2 . Hence, these DRIFTS spectra provide evidence for NO-to- NO_2 conversion on Mn-catalytic sites.

The aim of DFT atomistic modeling is to apply first principles to elucidate the likely reaction mechanism and possible intermediate species participating in the catalytic cycle. Here, we focus on the NO oxidation reaction occurring on the (110) surface of SmMn_2O_5 on the basis of information from HRTEM images. Weak NO and O_2 adsorption ($<0.24\text{ eV}$) was determined by DFT calculations on this ideal (110) surface, as shown in Fig. 2G. In addition, lattice oxygen is unlikely to react because oxygen-vacancy formation on this ideal surface requires 2.2 to 4.0 eV. Owing to the catalytic inertness of the ideal (110) surface, (110) stepped surfaces were investigated. DFT-based screening established that Mn-Mn dimers on the stepped surface (Fig. 2H) are responsible for activating oxygen molecules. Spontaneous dissociation of O_2 at the dimer sites leads

to surface mobile atomic oxygen with a diffusion energy barrier of 0.55 eV (supplementary text S-IX). Figure 4 displays a potential NO oxidation reaction route on the stepped mullite surface. On the O-covered Mn-Mn sites, incident NO (g) adsorbs on the O site to form Mn-nitrite ($-\text{NO}_2$). Monodentate Mn-nitrite ($-\text{NO}_2$) undergoes a transformation to bidentate Mn-nitrate ($-\text{NO}_3$) with an energy barrier of 0.72 eV. NO_2 is subsequently released via a distorted monodentate Mn-nitrate transition state by overcoming an energy barrier of 0.90 eV, leaving behind a bridged O atom. Two adjacent Mn-Mn sites with a bridged O energetically prefer to adsorb O_2 compared to NO by 0.66 eV. In the last step of the reaction cycle, O_2 gas molecules decompose into atomic O (en-

ergy barrier of 0.83 eV), thereby restoring the surface to fully O-covered Mn-Mn sites, which become available for the next cycle of NO conversion. As a result, we propose NO_2 desorption from manganese nitrates as the rate-limiting step with an energy barrier of 0.90 eV.

By comparison, the activation energy of NO oxidation on fresh Pt particles (12, 13) is 0.82 to 0.87 eV, but aged and sintered Pt displays a lower activity than aged MnCe-7:1. Moreover, the asymmetry of the SmMn_2O_5 NO oxidation curve in Fig. 1 suggests that there is NO_x inhibition to NO oxidation during ramp-up, corroborating that the rate-determining step involves NO_2 desorption. MnCe-7:1 shows less inhibition for the ramp-up and ramp-down cycles.

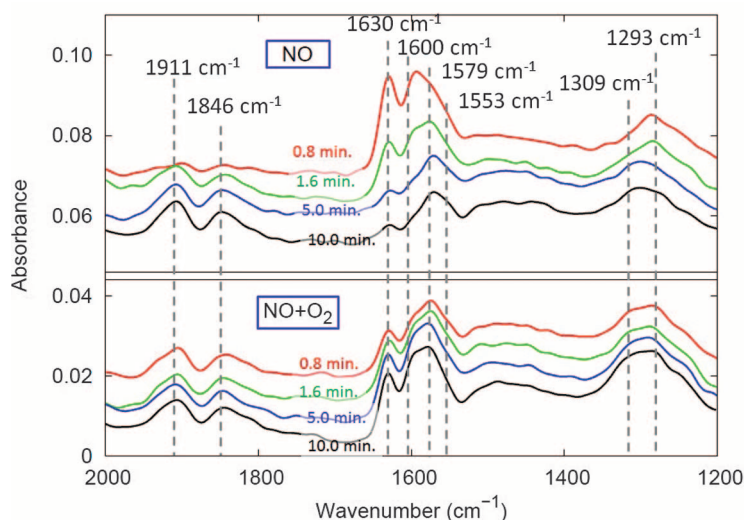


Fig. 3. Evolution of DRIFTS spectra at 200°C on MnCe 7:1 during exposure to NO and $\text{NO} + \text{O}_2$ feed gases with time.

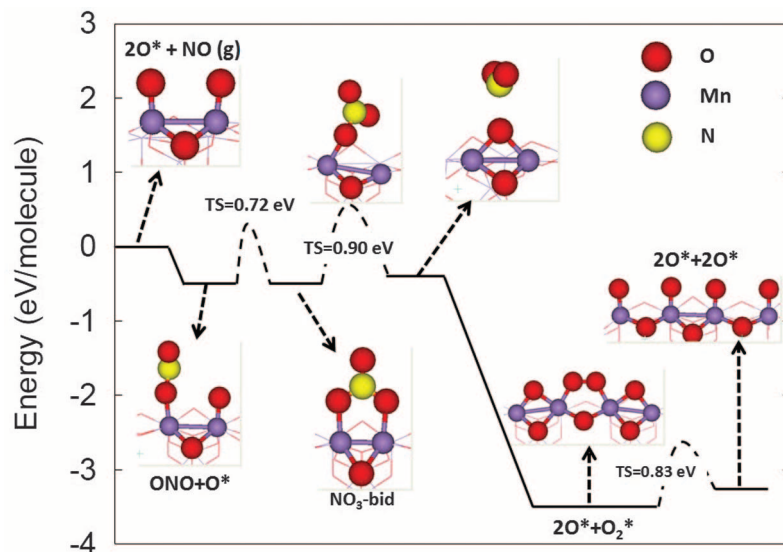


Fig. 4. Proposed reaction mechanism of NO conversion into NO_2 (g) on the stepped mullite (110) surface. TS, transition state.

Four key factors contribute to the overall catalytic activity of MnCe-7:1. Mn dimer sites are established as the main source of activity on SmMn_2O_5 . Addition of Sr increases the surface area, exposing more active sites. Ceria adsorbs and dissociates O_2 such that O^* can diffuse (14) to Mn-Mn dimers at the interface. Lastly, the stabilized spinel Mn_3O_4 phase results in additional high-temperature (330° to 350°C) catalytic activity. Deviating too far from the reported composition ($\text{Mn}_7\text{SrSmCeO}_{14.83}$) may result in lower catalytic activity. For instance, an excessive amount of Sr leads to poor NO conversion because of the potential formation of Sr-perovskite-based oxides.

To determine how MnCe-7:1 performs in simulated diesel exhaust, we added MnCe-7:1 as a top layer to a commercial-style PtPd diesel oxidation catalyst (materials and methods S-VII). Coated cores were aged and subjected to a gaseous mixture of NO, CO, CO_2 , C_3H_6 , C_3H_8 , *n*-decane, steam, and O_2 in He. The results show

that the additional MnCe-7:1 layer was not detrimental to CO and HC performance and that NO oxidation was enhanced (supplementary text S-X).

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Supplementary Materials

www.sciencemag.org/cgi/content/full/337/6096/832/DC1
Materials and Methods (S-I to S-VII)
Supplementary Text (S-VIII to S-X)
Fig. S1 to S6
Tables S1 to S3
References (15–19)

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UV Dosage Levels in Summer: Increased Risk of Ozone Loss from Convectively Injected Water Vapor

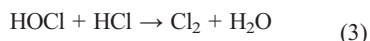
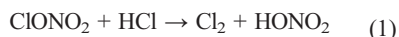
James G. Anderson,* David M. Wilmoth, Jessica B. Smith, David S. Sayres

The observed presence of water vapor convectively injected deep into the stratosphere over the United States can fundamentally change the catalytic chlorine/bromine free-radical chemistry of the lower stratosphere by shifting total available inorganic chlorine into the catalytically active free-radical form, ClO. This chemical shift markedly affects total ozone loss rates and makes the catalytic system extraordinarily sensitive to convective injection into the mid-latitude lower stratosphere in summer. Were the intensity and frequency of convective injection to increase as a result of climate forcing by the continued addition of CO_2 and CH_4 to the atmosphere, increased risk of ozone loss and associated increases in ultraviolet dosage would follow.

The occurrence, evolution, and cause of dramatic stratospheric ozone losses over the Antarctic in winter/early spring have been documented for three decades (1–9). Large ozone losses over the Arctic in the Northern Hemisphere winter/early spring (10–12) also have been reported and analyzed, with the largest ozone loss ever recorded over the Arctic occurring in 2011, rivaling losses observed over the Antarctic (13). Here, we present a chain of evidence that addresses the probability of increased ozone loss over mid-latitudes of the Northern Hemisphere motivated by a series of observations of the convective injection of water

vapor into the stratosphere over the United States in summer.

Large ozone losses that occur over the polar regions result directly from heterogeneous reactions involving inorganic chlorine:



These reactions serve primarily to transform inorganic chlorine (principally HCl and ClONO_2 , which constitute ~97% of available inorganic chlorine) into the rapidly photolyzed intermediates Cl_2 and HOCl, followed by reaction of the product Cl atoms with ozone to form the primary catalytically active chlorine radical ClO. This shift of inorganic chlorine to free-radical form ("chlorine activation") then establishes,

under conditions of solar illumination, a new photochemical steady state between ClO, ClOOCl , ClONO_2 , and HCl.

Of key concern is the temperature at which the above triad of heterogeneous reactions begins to become important [(14, 15) and citations therein]. Examination of conditions in the Arctic lower stratosphere coupled with emerging results from laboratory experiments have shown that the dominant pathway for chlorine activation appears to be on cold sulfate-water aerosols (14–18). Thus, it is both temperature and water vapor in combination with simple binary sulfate-water aerosols that primarily determine the kinetics for rapid chlorine activation.

The relationship that defines the threshold for rapid activation of chlorine as a function of the combination of temperature, water vapor mixing ratio, and aerosol reactive surface area for a solar zenith angle (45°) representative of mid-latitude summer conditions is summarized in Fig. 1A. The threshold for activation is defined as the point at which net chlorine activation exceeds a 10% conversion of inorganic chlorine to free-radical form in the initial diurnal cycle after convective injection of water vapor. The rate of chlorine activation is calculated by using the complete kinetic model for the triad of heterogeneous reactions (Eqs. 1 to 3), using the formulation of Shi *et al.* (14), as well as the relevant photochemical reaction kinetics discussed subsequently. As the water vapor concentration increases, so too does the threshold temperature for chlorine activation. As sulfate aerosol surface area increases, the temperature necessary for chlorine activation increases further. For example, at a commonly observed, unperturbed, stratospheric water vapor mixing ratio of 5 parts per million by volume (ppmv) and aerosol surface area of $2 \mu\text{m}^2/\text{cm}^3$, the threshold temperature for activation is ~198 K. But at a water vapor mixing ratio of 12 ppmv

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and aerosol surface area of $2 \mu\text{m}^2/\text{cm}^3$, the threshold temperature increases by ~ 5 K, substantially increasing the probability of heterogeneous chlorine activation because temperatures in the lower stratosphere over the mid-latitude Northern Hemisphere in summer frequently lie in the range of 200 to 205 K.

Because the binary sulfate-water aerosols are ubiquitous in the lower stratosphere, if the necessary temperature and water conditions are met then heterogeneous conversion of inorganic chlorine to free-radical form can occur anywhere, not just in the polar regions. Although the Arctic lower stratosphere is marginally colder than is the mid-latitude lower stratosphere over the United States in summer, what matters is the combination of water vapor concentration and temperature.

The in situ observations of H_2O obtained from both the high-altitude NASA ER-2 and WB-57 aircraft extending over a number of recent missions are summarized in Fig. 1B. The data shown were retrieved during flights originally selected to observe the outflow from typical convective storms over the United States in summer. What proved surprising is the remarkable altitude to which large concentrations of water vapor are observed to penetrate. The convective injection of water into the stratosphere was also observed with surprising frequency, occurring in $\sim 50\%$ of

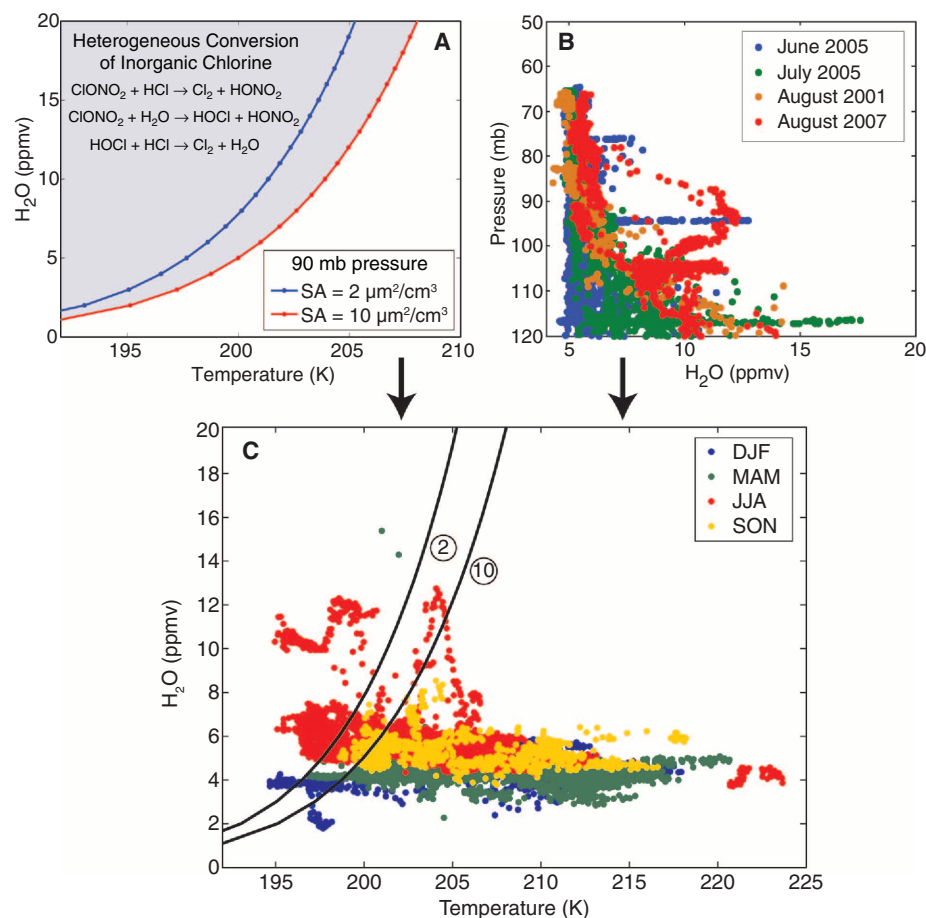
the summertime flights over the United States. The convective origin of this water vapor is established by simultaneous in situ observations of H_2O and the HDO isotopologue (19, 20), the concentration of which differentiates between direct convective injection and other pathways linking the troposphere and stratosphere (19, 21–23). The observed presence of water vapor enhancements reaching and occasionally exceeding 12 ppmv at temperatures in the vicinity of 200 K in the altitude region between 15 and 20 km, as displayed in Fig. 1B, may have important consequences.

The initiation of fundamental changes in the photochemistry of the lower stratosphere in summer is captured in Fig. 1C, in which the observed in situ H_2O mixing ratios and temperatures at 90 ± 10 mbar pressure are superimposed on the threshold plot for chlorine activation over the range of 2 to $10 \mu\text{m}^2/\text{cm}^3$ for reactive surface area. It is clear that at observed water vapor concentrations and temperatures, the threshold for chlorine activation converting inorganic chlorine to free-radical form is routinely crossed in the summertime. The result is that ClO can become a major component of the available inorganic chlorine budget within regions of high water vapor. Convective injection of water vapor to heights reported here can occur in storm systems that are ~ 50 km across, with smaller domains of high-

altitude injection imbedded within them at their origin (24). The elevated concentrations of water can spread to 100 km or more in horizontal extent within a few days and remain at the elevated levels reported here over a period of days (19, 25). This phenomenon has been analyzed by Newman *et al.* (26) by using high-altitude (70 to 100 mbar) observations of rocket plume dispersion that defines the rate of horizontal spreading from a point source. Additionally, the circular flow pattern of air in the lower stratosphere over the United States resulting from the North American summer monsoon provides the potential for repeated convective injection events into the summer lower stratosphere over the United States.

In order to address the photochemical catalytic free-radical chemistry that controls the loss rate of ozone in the lower stratosphere, we used extensive observations of the free-radical photochemistry under unperturbed conditions (in the absence of convectively injected H_2O) in the mid-latitude lower stratosphere developed over nearly a decade of simultaneous in situ observations of OH, HO_2 , ClO, BrO, NO, NO_2 , HCl, H_2O , and O_3 and the important tracers CO_2 , N_2O , and CH_4 [for example, (27)]. We also draw heavily on studies that followed in the vicinity of and within the Arctic vortex during conditions before, during, and after rapid ozone loss (28–32)

Fig. 1. The lines of evidence leading to the conclusion that observed combinations of high water vapor concentrations and low temperatures over the United States lead to the conversion or activation of inorganic chlorine to free-radical form on cold sulfate-water aerosols. (A) The regime of water vapor and temperature for the triad of heterogeneous reactions responsible for rapid chlorine activation under sunlight conditions in the mid-latitude lower stratosphere is identified for two aerosol surface areas. (B) Observations of water vapor in the summertime over the United States show numerous occurrences of elevated concentrations reaching pressure altitudes deep into the stratosphere. Invariably, regions high in water vapor are found to be high in the isotopologue HDO, demonstrating that the high water vapor concentrations result from direct convective injection. (C) Observations of water vapor mixing ratios and temperatures at 90 ± 10 mbar color-coded by months, superimposed on a plot of the threshold conditions for chlorine activation at surface area 2 and $10 \mu\text{m}^2/\text{cm}^3$.



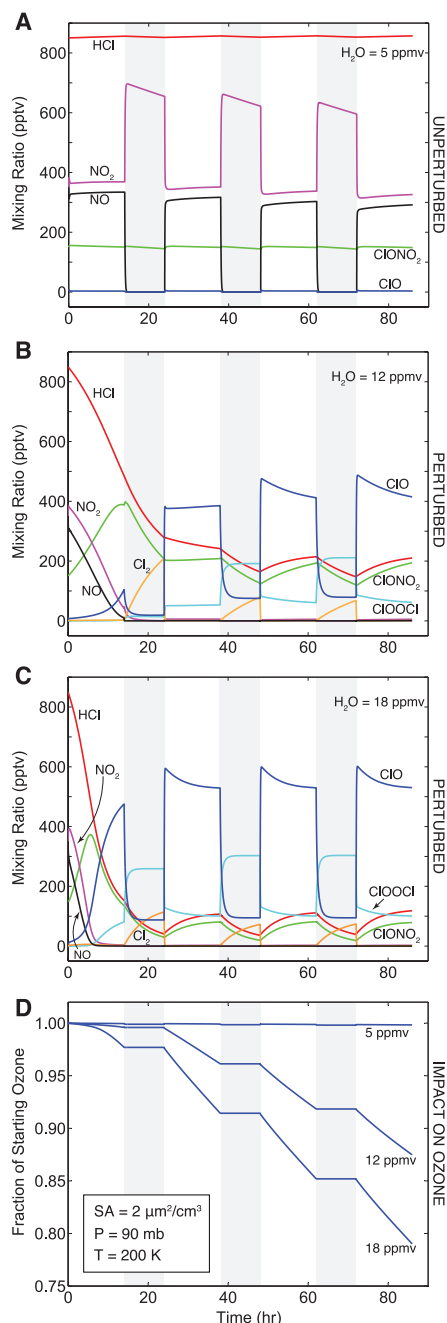


Fig. 2. The impact of convectively injected water vapor into the Northern Hemisphere mid-latitude stratosphere in summer is summarized in three stages. **(A)** Calculation of the time dependence of HCl, ClONO₂, ClOOCl, NO, NO₂, Cl₂, and ClO under unperturbed conditions in the summertime lower stratosphere (90-mbar pressure) for which 5 ppmv H₂O, 200 K, and 2 μm²/cm³ are appropriate. **(B)** Time evolution of the same species over the four diurnal periods after convective injection of water vapor to 12 ppmv. **(C)** Time evolution of the same species but with a water vapor mixing ratio of 18 ppmv after convective injection. **(D)** Loss of ozone over the four diurnal periods for 5, 12, and 18 ppmv of water vapor. Daytime calculations are run at a solar zenith angle of 45°; regions shaded in gray represent darkness.

that included measurements of ClONO₂ and ClOOCl in addition to the species listed above. These analyses provide strict tests for the completeness of the photochemical reaction sets used to calculate the concentrations of the important species, as well as the reaction rate constants, and photodissociation cross sections. We used the latest Jet Propulsion Laboratory evaluation (33) for the network of reactions under unperturbed conditions, including the heterogeneous reactions on cold binary sulfate-water aerosols (14), to calculate the impact of elevated water vapor concentrations on the heterogeneous processing of inorganic chlorine. For simplicity, the volume element in the calculations presented here is fixed at a selected pressure altitude and temperature; daylight is represented by a fixed solar zenith angle of 45°. The model incorporates the standard reactions necessary to provide a comprehensive treatment of the evolution of NO_y, Cl_y, Br_y, and HO_x species that includes 62 photochemical reactions and 31 molecular species. We present the results by displaying the evolution of the key species with time by contrasting the mixing ratios of HCl, ClONO₂, Cl₂, NO₂, NO, ClOOCl, and ClO for three conditions.

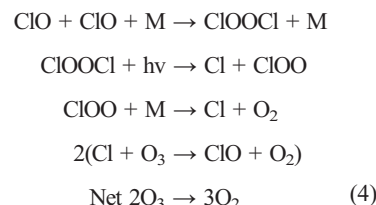
As shown in Fig. 2A, we tracked the evolution with time for summer, mid-latitude conditions over four 14-hour segments of daylight, with three intervening 10-hour periods of darkness for conditions of 90-mbar pressure, a temperature of 200 K, a water vapor mixing ratio of 5 ppmv, and a typical reactive surface area of 2 μm²/cm³. As demonstrated in Fig. 2A, under these conditions the key inorganic chlorine species HCl and ClONO₂ are unaffected by the heterogeneous reactions on cold sulfate-water aerosols—nor are the NO, NO₂, and ClO concentrations affected under these unperturbed conditions in the lower stratosphere. The concentrations and ratios of HCl to ClONO₂ and NO to NO₂ are consistent with observations under unperturbed conditions, as are the concentrations of the other 27 species in the calculation, including the catalytic free radicals.

In Fig. 2B, we held all conditions the same as in Fig. 2A, except we increased the mixing ratio of H₂O to that observed repeatedly in regions influenced by convective injection: 12 ppmv. The impact of crossing the threshold for chlorine activation is clear, even on the time scale of just 4 days of exposure to solar illumination. The case for 18 ppmv water, shown in Fig. 2C, represents a reasonable estimate of the initial water vapor mixing ratio after convective injection, as the region of enhanced water vapor in Fig. 1C mixed horizontally over 2.5 days before being intercepted by the aircraft. The contrast between 5 ppmv (the unperturbed case) and the cases for 12 ppmv and 18 ppmv highlights the sensitivity of the reaction system to injected water vapor.

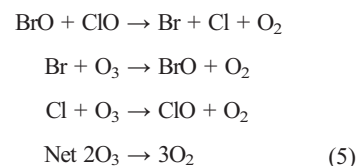
We can summarize the sequence of events after injection of water vapor. In the first diurnal period, the heterogeneous reactions on cold

sulfate-water aerosols begin to rapidly convert HCl and ClONO₂ to Cl₂ and HOCl. The Cl₂ and HOCl photodissociate quickly to Cl atoms, which then react with O₃ to produce ClO. The ClO thus produced reacts with NO₂ to form ClONO₂, effectively shifting available inorganic chlorine from HCl to ClONO₂. Simultaneously, the reaction of ClONO₂ with HCl in effect converts NO₂ to HONO₂. Because NO and NO₂ interconvert on the time scale of minutes, the removal of NO₂ draws down the concentration of NO in unison. As NO₂ is diminished, ClO begins to increase because its conversion to ClONO₂ begins to slow. With the termination of sunlight in the first diurnal period, Cl₂ begins to build up rapidly, fed by the heterogeneous reactions but without removal by photodissociation. The heterogeneous reactions continue to remove HCl and ClONO₂, forming Cl₂, HOCl, and HONO₂ in the dark. With sunrise on the second day, a rapid increase in ClO occurs as a result of Cl₂ photolysis followed by reaction of Cl with O₃. The rate of increase of ClO and ClOOCl depends on the concentration of water vapor, as the contrast between Fig. 2, B and C, makes clear. With the reinitiation of sunlight on the third day, the pattern of highly amplified ClO and ClOOCl in steady state with ClONO₂ is established, in combination with slow production of HCl from the reaction of Cl with CH₄ and of ClO with OH.

The impact on ozone loss rates for the perturbed cases displayed in Fig. 2, B and C, evolves rapidly over the same time scale. During the first diurnal period, control of the catalytic destruction rate of ozone shifts to a combination of (i) the ClO dimer catalytic cycle introduced by Molina and Molina (34):



and (ii) the bromine-controlled catalytic cycle introduced by McElroy *et al.* (3) and subsequently analyzed by Salawitch *et al.* (35):



As a result, the catalytic loss of ozone can increase by two orders of magnitude over that for the unperturbed case within the horizontal and vertical extent of the region of injected water vapor.

In Fig. 2D, we contrast the fractional removal of ozone as a function of time after convective injection of water for the cases of 12 and

18 ppmv water compared with the unperturbed case. For the case of 12 ppmv water, ~13% of the ozone is lost after four sunlight periods, and the loss rate of ozone is ~4% per day in the following few diurnal periods. For the 18 ppmv case, ~21% of the ozone is lost after four sunlight periods, with a loss rate of ~6% per day in the following few diurnal periods. Therefore, the catalytic destruction of ozone within the domain of high water vapor concentration has taken full control of the ozone loss rate and dominates the local photochemical production rate (and the rate of transport-controlled replenishment of ozone) by two orders of magnitude. Once inorganic chlorine has been activated to free-radical form, several days are required for the reformation of HCl and ClONO₂ after the water vapor concentration has dropped below ~8 ppmv. However, because the kinetics of heterogeneous chlorine activation also depends on temperature, and water vapor radiatively cools the lower stratosphere, ozone loss rates can persist.

We emphasize that although the quantitative details of the calculations shown in Fig. 2 will vary with temperature, aerosol surface area, pressure, inorganic chlorine, and duration of elevated water vapor, the key point here is the potential for significant chlorine activation and ozone loss when water vapor is elevated. As exemplified by the in situ observations presented in Fig. 1B, there is a growing realization that convective moistening occurs in the lower stratosphere over the central United States during the summer (19, 36–38). Two instances of elevated ClO at mid-latitudes have been reported, both near the tropopause region under conditions of elevated H₂O (39, 40). There is also growing recognition of the potential link between the forcing of climate by increasing concentrations of CO₂, CH₄, and other infrared active gasses and convective injection of water over the United States (41, 42). Again, what is remarkable is the depth to which these convective events penetrate into the stratosphere coupled with the steep gradients of increasing (i) inorganic chlorine, (ii) free-radical concentrations, and (iii) ozone concentrations with altitude into which this water is injected. The altitude interval between 15 and 20 km that is affected by this convective injection contains ~20% of the ozone column in the summertime over the United States.

As a result of the separation of NASA airborne missions into either (i) a chemistry/ozone-loss focus or (ii) a climate/dynamics/radiation focus, the in situ free-radical instruments for OH, HO₂, NO₂, ClO, BrO, ClONO₂, and ClOOCl have never been deployed into regions of convectively injected water vapor over mid-latitudes of the Northern Hemisphere, and so these measurements are lacking.

There are a number of important considerations associated with the issue of convective injection of water vapor inducing chlorine activation and catalytic removal of ozone over mid-latitudes of the Northern Hemisphere in summer. First is

that a remarkably dry stratosphere characterizes the current climate state. However, the paleo-record holds evidence that the stratosphere, under conditions of high CO₂ concentrations, was characterized by substantially higher water vapor concentrations than is the case today (43, 44). If currently increasing concentrations of CO₂, CH₄, and other infrared active gasses force the stratospheric system to a state of increasing water vapor concentrations, the impact on ozone is of considerable concern given the concentrations of chlorine and bromine in the stratosphere today.

Second, the loss of ice from the Arctic Ocean opens the possibility for substantial increases in CO₂ and CH₄ release from melt zones in the Arctic. A release of just 0.5% per year of the carbon tied up in the soils of Siberia and northern Alaska alone will double the carbon added to the atmosphere each year from the combustion of fossil fuels worldwide (45). This release of carbon from clathrates and permafrost will accelerate the forcing of the climate that is potentially linked to the intensity and frequency of convective injection of water into the stratosphere.

Third, engineering the climate by the addition of sulfates to increase aerosol concentrations and reduce climate forcing by reflecting sunlight back to space (46, 47) would significantly increase reactive surface area, which would accelerate the processing of chlorine to free-radical form (Fig. 1, A and C), decreasing ozone concentrations. In the same vein, the convective injection of water vapor into the stratosphere increases the sensitivity of ozone loss to volcanic injection of sulfates into a stratosphere with current loading of chlorine and bromine. Evidence for this was presented by Salawitch *et al.* (35) for the eruption, in 1991, of Mount Pinatubo.

Fourth, from the perspective of human health a primary concern is that decreasing ozone concentrations, particularly in summer over populated areas, results in increased ultraviolet (UV) dosage levels. Sustained increases in UV dosage levels are in turn associated with the increased incidence of skin cancer (48, 49), which is currently 1 million new cases a year in the United States (49).

Last, we emphasize that because chlorine activation depends exponentially on water vapor and temperature, and in turn that the forcing of climate may well control the convective injection of water into the lower stratosphere, the idea that ozone “recovery” is in sight because we have controlled chlorofluorocarbons and halon release is a potential misjudgment.

We have focused in this paper on conditions in the summer lower stratosphere over the United States because that is the region for which we have direct experimental evidence of deep convective injection. Similar conditions may hold elsewhere, for example over the Tibetan plateau, north of the Asian Monsoon.

The point is not that we know exactly when ozone will begin to decrease over the mid-latitude

Northern Hemisphere in summer if convective injection of water continues or increases, but rather that (i) the photochemical system controlling the concentration of ozone in the stratosphere is extraordinarily sensitive to the convective injection of water vapor that occurs over populated areas in summer, (ii) the response of ozone to chlorine dimer and chlorine/bromine catalytic cycles is extremely sensitive to the altitude of penetration of convective injection, and (iii) were the intensity and frequency of these convective events to increase irreversibly as a result of climate forcing by the continued addition of CO₂ and CH₄ to the atmosphere, decreases in ozone and associated increases in UV dosage would also be irreversible.

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Circadian Rhythm of Redox State Regulates Excitability in Suprachiasmatic Nucleus Neurons

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Daily rhythms of mammalian physiology, metabolism, and behavior parallel the day-night cycle. They are orchestrated by a central circadian clock in the brain, the suprachiasmatic nucleus (SCN). Transcription of clock genes is sensitive to metabolic changes in reduction and oxidation (redox); however, circadian cycles in protein oxidation have been reported in anucleate cells, where no transcription occurs. We investigated whether the SCN also expresses redox cycles and how such metabolic oscillations might affect neuronal physiology. We detected self-sustained circadian rhythms of SCN redox state that required the molecular clockwork. The redox oscillation could determine the excitability of SCN neurons through nontranscriptional modulation of multiple potassium (K⁺) channels. Thus, dynamic regulation of SCN excitability appears to be closely tied to metabolism that engages the clockwork machinery.

Circadian rhythms coordinate body systems and synchronize the internal milieu with daily and seasonal changes in light on Earth. Diurnal changes in the environment generate daily fluctuations in energy availability, to which internal metabolic systems are tuned by

self-sustained circadian clocks (1, 2). These near-24-hour rhythms emerge from transcriptional-translational feedback loops of core clock genes (3) and oscillations in regulatory cytoplasmic elements, including adenosine 3',5'-monophosphate (cAMP) (4, 5), Ca²⁺ (6), and activity of protein kinases (7). Superimposed upon circadian rhythms of metabolism are near-24-hour oscillations due to the contingencies of life. At the cellular level, metabolic state is manifest as redox state. It is usually described by the homeostasis of reactive free radicals, such as nicotinamide adenine dinucleotide (NAD⁺) and flavin adenine dinucleotide (FAD), from reduction-oxidation reactions in metabolism (8). Circadian and energetic cycles are coupled through transcriptional modulation by core clock proteins of genes that regulate metabolism (9, 10), as well as the sensitivity of clock gene transcription to redox state (11–13). However, nontranscriptional interdependency of redox state and neuronal physiology in the circadian context is unexplored.

To determine whether redox state exhibits a daily rhythm in the brain's circadian clock, we performed ratiometric redox fluorometry by two-

photon laser microscopy of organotypic slices of suprachiasmatic nucleus (SCN)-bearing rat hypothalamus (14). The relative redox state was measured noninvasively from the ratio of autofluorescence emissions in response to 730-nm excitation of two cofactors of cellular metabolism, the oxidized form of FAD (500+ nm) and the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) (400+ nm) (15). We found an endogenous, near-24-hour oscillation of redox state in SCN tissue from wild-type rat and mouse ($\tau = 23.74 \pm 0.26$ (SEM) hours, $\tau = 23.75 \pm 0.30$ hours, respectively; χ^2 periodogram analysis, $N = 5$ brain slices) (Fig. 1, A, B, D, and E). Application of the oxidizing reagent, diamide (DIA, 5 mM), increased the FAD/NADPH ratio within 2 min [$\Delta F_{500+}/F_{400+} = 0.022 \pm 0.018$, $P < 0.05$, paired Student's t test, $N = 6$ brain slices] (fig. S1, A and B). On the other hand, exposure to a reducing reagent, glutathione (GSH, 1 mM), decreased the ratio ($\Delta F_{500+}/F_{400+} = -0.022 \pm 0.010$, $P < 0.01$, paired Student's t test, $N = 6$ brain slices) (fig. S1C). We further evaluated SCN slices from *Bmal1*^{−/−} mice, which lack circadian rhythms (16, 17). *Bmal1*^{−/−} SCNs exhibited stochastic, but not circadian, oscillations in relative redox state (Fig. 1, C and F) ($N = 5$ brain slices). Thus, circadian redox oscillations in rodent SCN require a functional molecular clockwork involving the clock gene, *Bmal1*.

To determine temporal phasing of the SCN redox oscillation, we evaluated two indicators of redox state. First, we examined points across the circadian cycle of SCN brain slices for glutathiolation, the capacity of proteins to incorporate reduced GSH, which binds to available disulfide bonds (18). Glutathiolation peaked in the early night, indicating a relatively oxidized state, and was lower in midday, indicating a relatively reduced state (Fig. 1, G and H) [$P < 0.05$, one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test, $N = 6$ experiments]. We next analyzed the ascorbic acid system, an important antioxidant and neuroprotective buffer in the brain (19). We used capillary electrophoresis with laser-induced fluorescence detection (CE-LIF) to directly measure the concentrations of dehydroascorbic acid (DHA) versus its reduced counterpart, ascorbic

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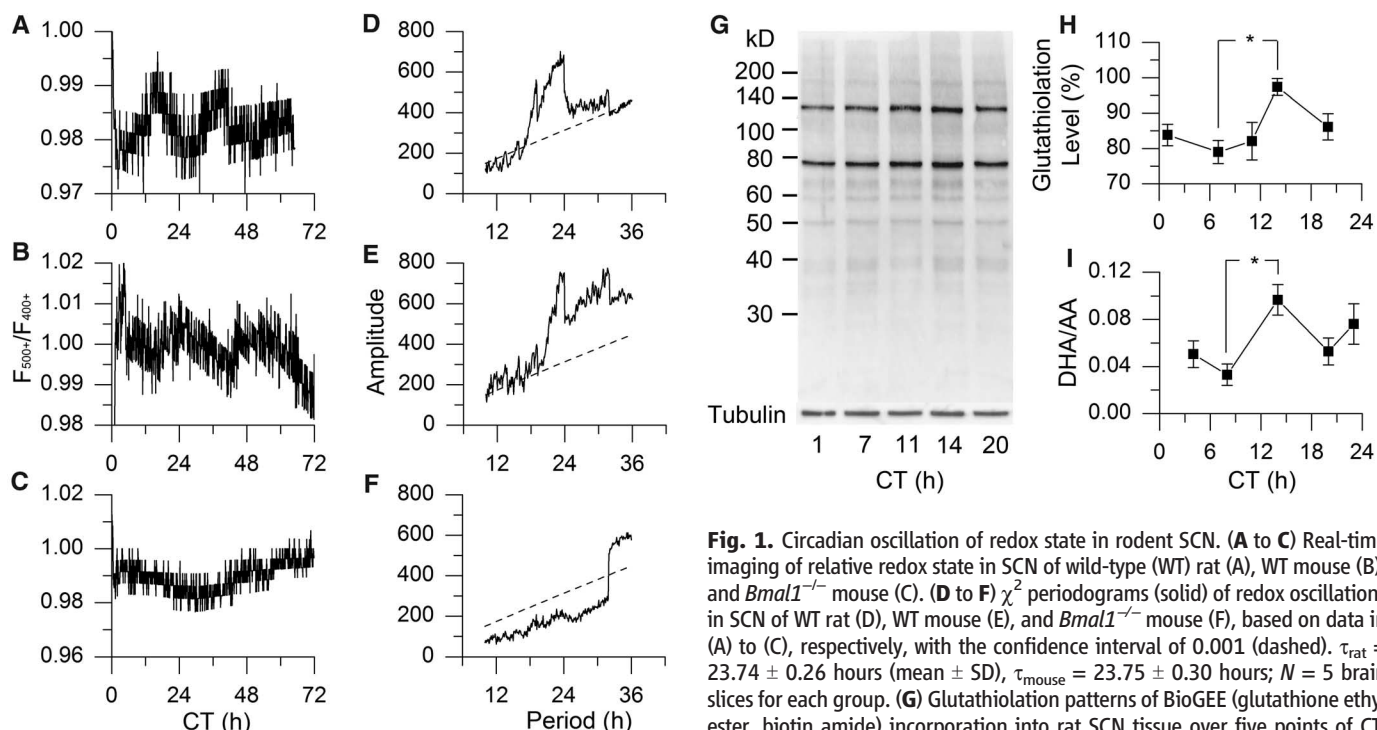
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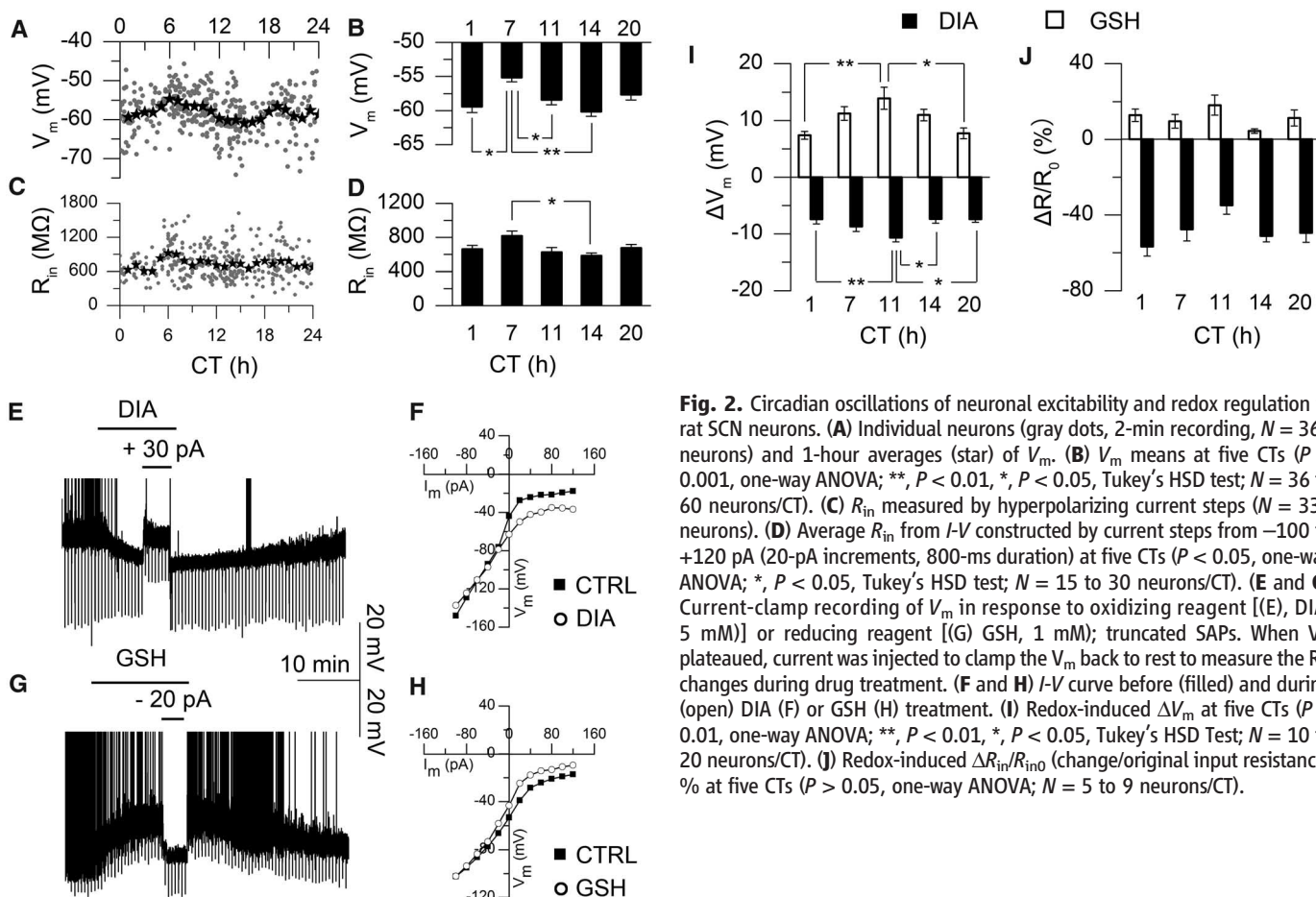
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Protein glutathiolation over five CTs in rat SCN ($P < 0.05$, one-way ANOVA; *, $P < 0.05$, Tukey's HSD test; $N = 6$ experiments on separate SCNs). (I) DHA/AA ratio in rat SCN over five CTs ($P < 0.05$, one-way ANOVA; *, $P < 0.05$, Tukey's HSD test; $N = 3$ experiments on separate SCNs).



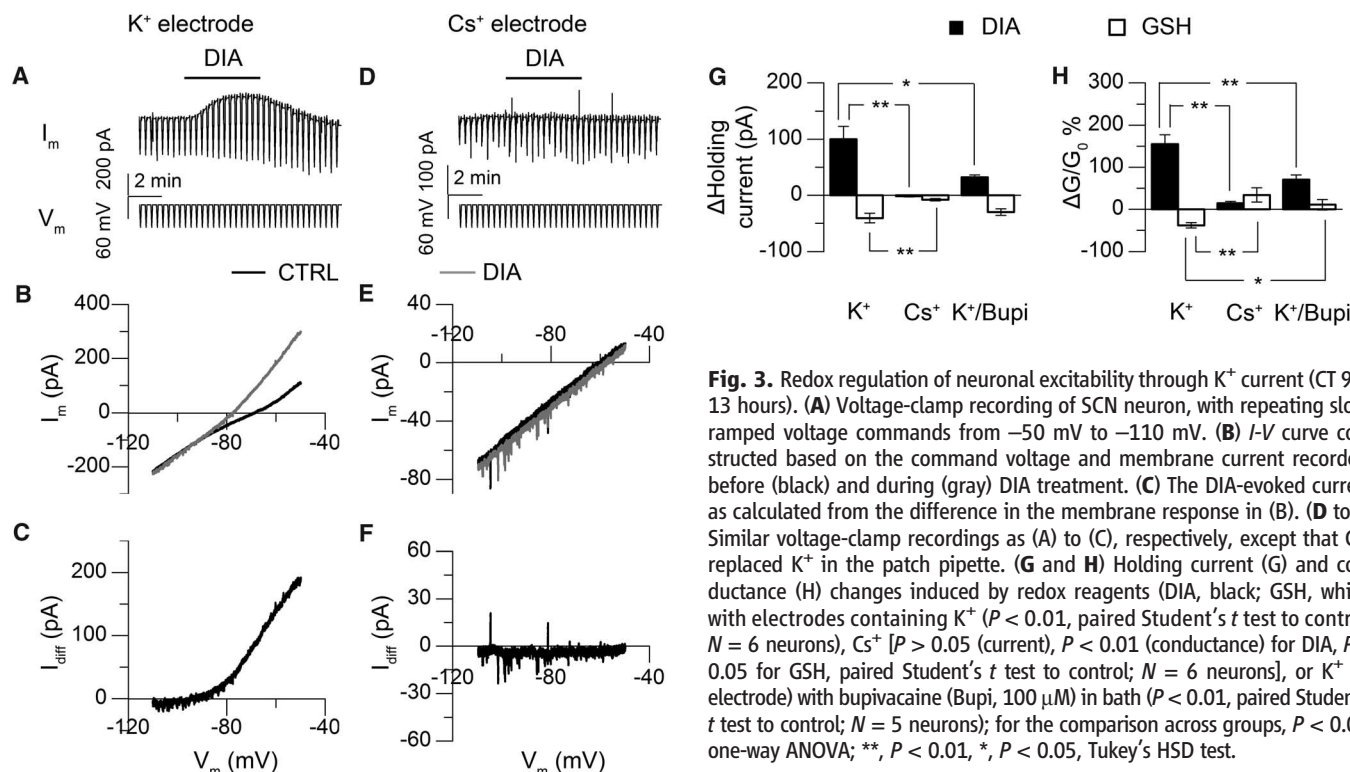


Fig. 4. Redox regulation of voltage-dependent K^+ currents (CT 9 to 13 hours).

(A) Recording protocol of repeating voltage-step commands to voltage-clamped SCN neurons (15), before, during, and after drug treatment. (B and C) Current responses to the voltage-step commands of -10 mV pulses, after either -90 mV (B) or -40 mV (C) prepulse, before (black) or during (red) DIA treatment. (D) Voltage-dependent outward current in response to -10 mV voltage-step stimulation, calculated from the difference between the current responses in (B) and (C). (E and F) Effects of 4-AP (5 mM) and TEA (20 mM) on outward current evoked by DIA. (G) Transient current (<10 ms) changes in response to redox treatment (DIA, black; GSH, white), with or without 4-AP or TEA ($P < 0.01$, one-way ANOVA; **, $P < 0.01$, Tukey's HSD test; $N = 5$ to 6 neurons/condition). (H) Persistent current (230 to 250 ms) changes in response to redox treatment, with or without 4-AP or TEA ($P > 0.05$, one-way ANOVA; $N = 5$ to 6 neurons/condition).

acid (AA) (20). Amounts of DHA/AA oscillate with a circadian rhythm, similar to glutathiolation: DHA/AA is highest in the early night and lowest in midday (Fig. 1I) ($P < 0.05$, test as above, $N = 3$ experiments). Parallel changes in these distinct redox systems confirm circadian oscillation of global redox state in rat SCN, with a significantly oxidized state in the early night versus a reduced state during the daytime. These results support and extend the redox oscillation found in peripheral tissue (21, 22) to the central circadian clock in the brain.

To assess possible relations between the circadian oscillations of redox state and neuronal physiology, we evaluated membrane excitability in rat SCN neurons by examining resting membrane potential (V_m), input resistance (R_m), and spontaneous action potentials (SAP). Recording from current-clamped neurons, we observed circadian oscillations of V_m [Fig. 2A, $N = 364$ neurons; Fig. 2B, $N = 36$ to 60 neurons/CT at five circadian times (CTs) around the free-running clock cycle, $P < 0.001$, test as above], R_m (Fig. 2C, $N = 337$ neurons; Fig. 2D, $N = 15$ to 30 neurons/CT, $P < 0.05$, test as above), frequency of SAP (fig. S2A, $N = 334$ neurons), and percentage of neurons discharging SAP (active, fig. S2C, $N = 334$ neurons) (15). These data from rats exhibit a similar circadian pattern in membrane excitability as from mice (23). Comparing circadian oscillations of redox state (Fig. 1, H and I) with V_m in SCN neurons (Fig. 2B) revealed that the daytime reduced state matched the depolarized V_m , whereas the oxidized state of subjective night aligned with hyperpolarized V_m .

To probe potential interdependency of redox state and neuronal excitability, we tested the effects of pharmacological redox manipulation on V_m of SCN neurons at various CTs. The oxidizing reagent, DIA, hyperpolarized V_m in a reversible manner (Fig. 2E, -8.68 ± 0.64 mV, mean around circadian cycle, $N = 104$ neurons); in contrast, exposure to the reducing reagent, GSH, caused depolarization (Fig. 2G, $+10.67 \pm 1.03$ mV, $N = 97$ neurons). Similar results were obtained from current-clamp recording with redox reagents in the patch pipette (fig. S4). Exogenous redox regulation of V_m in SCN depends on CT: both drugs caused maximal effects near subjective dusk (CT 10 to 12 hours) and minimal effects near subjective dawn (CT 0 to 2 hours, Fig. 2I, $P < 0.01$, test as above, $N = 10$ to 20 neurons/CT). Shifts in V_m caused by redox manipulation were associated with changes of R_{in} . Based on the slopes of current-voltage (I - V) curves constructed before and during redox treatment, DIA was found to decrease the R_{in} of SCN neurons by 364 ± 30 M Ω (48.83%, Fig. 2, F and J, $N = 41$ neurons), whereas GSH increased R_{in} by 64 ± 11 M Ω (10.81%, Fig. 2, H and J, $N = 36$ neurons). Percentage changes in R_{in} were not correlated with changes in V_m (fig. S5, $P > 0.05$, linear correlation and regression, $N = 37$ to 42 neurons) and were independent of CT (Fig. 2J, $P > 0.05$, one-way ANOVA, $N = 5$ to 9 neurons/CT). Redox-induced changes in membrane properties were rapid, occurring in <2 min (68.7 ± 10.6 s).

To identify potential targets for redox regulation of neuronal excitability, we gradually changed command voltages from -50 to -110 mV (6-s duration) on voltage-clamped SCN neurons between CT 9 and 13 hours. By comparing membrane currents before and during exposure to the redox reagents, we examined the voltage dependency of target ion channels. Bath application of DIA elicited a strong outward current (100.1 ± 22.8 pA at -50 mV, $N = 6$ neurons) (Fig. 3, A, B, C, and G), associated with an increased conductance as indicated by a greater slope of the current response to the ramped voltage command. The I - V relationship before and during DIA treatment revealed a reversal potential at -81.4 ± 7.0 mV and conductance increases of $154.9 \pm 22.5\%$ (Fig. 3, B and H). Thus, DIA appears to act through an outward-rectifier K^+ channel (Fig. 3C). We confirmed this prediction by replacing K^+ with Cs^+ , a general K^+ -channel blocker, in the recording pipette. Under this condition, the DIA-evoked outward current was significantly attenuated (-1.6 ± 1.2 pA at -50 mV, $P < 0.01$, Tukey's HSD Test, $N = 6$ neurons) (Fig. 3, D to G). Only small conductance changes could be detected during DIA treatment ($14.2 \pm 4.7\%$) (Fig. 3, E and H). Exposure to GSH elicited an inward current in SCN neurons (-40.6 ± 8.5 pA at -50 mV, $N = 6$ neurons/condition) (Fig. 3G), with a reversal potential at -79.9 ± 4.0 mV and a conductance change of $-38.2 \pm 6.3\%$ (Fig. 3H).

The GSH-evoked inward current was attenuated by Cs^+ in the internal solution, as well (-7.9 ± 2.1 pA at -50 mV, $34.1 \pm 16.8\%$ conductance changes, $P < 0.01$, test as above, $N = 6$ neurons/condition) (Fig. 3, G and H), also supporting the involvement of K^+ currents.

To explore the potential role of leak K^+ channels in redox regulation, we applied a specific blocker of this channel, bupivacaine (Bupi, 100 μ M). In the presence of Bupi, DIA induced an outward current of 31.9 ± 4.4 pA at -50 mV (Fig. 3G) ($N = 5$ neurons/condition), with conductance changes of $70.4 \pm 11.2\%$ (Fig. 3H) ($N = 5$ neurons), but amplitudes were lower than those in control media (Fig. 3, G and H) ($P < 0.05$, test as above). Bupi attenuated GSH-induced inward current and conductance changes, as well (-40.6 ± 8.5 pA at -50 mV, $11.0 \pm 12.4\%$, $P < 0.05$, test as above, $N = 5$ neurons/condition) (Fig. 3, G and H). These results support the leak K^+ channel as a target of redox regulation.

We further used voltage-step commands to examine the possibility that redox state regulates voltage-gated K^+ channels (Fig. 4, A to C) (15). We found that DIA significantly enhanced the transient peak of the outward current induced by -10 mV steps (288.4 ± 36.5 pA, $N = 5$ neurons) (Fig. 4, D and G). This enhancement was completely abolished by 4-aminopyridine (4-AP, 5 mM), a selective inhibitor of A-type K^+ channel (-11.7 ± 17.2 pA, $P < 0.01$, test as above, $N = 6$ neurons) (Fig. 4, E and G), but was insensitive to tetraethylammonium (TEA, 20 mM), a delayed rectifier K^+ -channel blocker (282.4 ± 34.7 pA, $P > 0.05$, test as above, $N = 6$ neurons) (Fig. 4, F and G). The persistent outward current was insensitive to DIA treatment with or without 4-AP or TEA ($P > 0.05$, one-way ANOVA, $N = 5$ to 6 neurons) (Fig. 4H). On the other hand, GSH suppressed the transient peak of the outward current (-149.5 ± 37.3 pA, $N = 5$ neurons) (Fig. 4G); similar to DIA, the suppression was sensitive to 4-AP (6.8 ± 14.3 pA, $P < 0.01$, Tukey's HSD Test, $N = 5$ neurons) (Fig. 4G) but not TEA (-164.8 ± 25.4 pA, $P > 0.05$, test as above, $N = 6$ neurons) (Fig. 4G), whereas the persistent outward current was not affected by any drug ($P > 0.05$, one-way ANOVA, $N = 5$ to 6 neurons/condition) (Fig. 4H). These results support the involvement of a 4-AP-sensitive voltage-gated K^+ channel in redox regulation.

The daily rhythm of electrical activity in the SCN is essential for the functionality of the central pacemaker in synchronizing the body clocks (24, 25); several K^+ channels have been identified underlying the changing excitability (26–28). We found that a redox regulation of K^+ conductance underwent circadian changes in SCN neurons, with characteristics of both leak and A-type K^+ channels. This provides a nontranscriptional pathway for the metabolic cycle to engage the clockwork machinery (fig. S6). Energetic fluctuation in the central nervous system has been considered to be a consequence of neuronal activity. However, our study implies that changes in cel-

lular metabolic state could be the cause, rather than the result, of neuronal activity. Cross talk between energetic and neuronal states bridges cellular state to systems physiology.

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Supplementary Materials

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Materials and Methods
Supplementary Text
Figs. S1 to S6
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Identification of the Cdc48•20S Proteasome as an Ancient AAA+ Proteolytic Machine

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Proteasomes are the major energy-dependent proteolytic machines in the eukaryotic and archaeal domains of life. To execute protein degradation, the 20S core peptidase combines with the AAA+ ring of the 19S regulatory particle in eukarya or with the AAA+ proteasome-activating nucleotidase ring in some archaea. Here, we find that Cdc48 and 20S from the archaeon *Thermoplasma acidophilum* interact to form a functional proteasome. Cdc48 is an abundant and essential double-ring AAA+ molecular machine ubiquitously present in archaea, where its function has been uncertain, and in eukarya where Cdc48 participates by largely unknown mechanisms in diverse cellular processes, including multiple proteolytic pathways. Thus, proteolysis in collaboration with the 20S peptidase may represent an ancestral function of the Cdc48 family.

In all domains of life, protein-unfolding machines fueled by adenosine triphosphate (ATP) and self-compartmentalized peptidases carry out intracellular protein degradation (1). The unfoldases belong to the AAA+ superfamily [adenosine triphosphatases (ATPases) associated with a variety of cellular activities] and function as hexameric rings. The barrel-shaped peptidases have an internal chamber containing the proteolytic active sites. All AAA+ proteases share common operating principles, including coaxial unfoldase-peptidase docking and translocation of unfolded substrates through the axial pores into the degradation chamber. The major degradation machine in eukaryotes is the 26S proteasome, consisting of the 20S core peptidase and 19S regulatory particles, which contain AAA+ Rpt₁₋₆ unfolding rings (2, 3). The 20S peptidase, which has an α - β - γ - α - β - γ architecture, is also ubiquitous in archaea, where it functions with the hexameric AAA+ proteasome-activating nucleotidase (PAN) (4). Paradoxically, PAN is absent from some archaea (table S1) and is not required for viability of other archaea in which 20S is essential (5). Thus, we reasoned that an unidentified pathway for proteasomal protein degradation must exist.

Cdc48/p97 is a double-ring AAA+ machine (6) that is highly conserved but has no clear function in archaea. By contrast, eukaryotic Cdc48 and specific adaptor proteins participate in diverse cellular processes, including proteolytic pathways, but by largely unknown molecular mechanisms (6). It is intriguing that many archaeal and eukaryotic Cdc48 enzymes have C-terminal HbYX (hydrophobic residue, tyrosine, any residue) motifs, which are also present in PAN and Rpt₁₋₆ subunits and dock into binding pockets on the 20S α ring (7).

The single AAA+ module of PAN is highly homologous to both AAA+ modules of Cdc48, but these proteins have distinct N domains (Fig. 1A) (8, 9). Searches of complete archaeal genomes revealed that ~15% contained no PAN genes but did contain 20S genes and at least one Cdc48 gene encoding an HbYX or related C-terminal sequence (Fig. 1B and table S1). Docking the crystal structures of *Thermoplasma acidophilum* 20S and mouse Cdc48/p97 showed that the C-terminal tails could be positioned to interact with the HbYX-binding clefts in the 20S α ring (fig. S1).

For interaction studies, we used *T. acidophilum* His₆-Cdc48^{EQ/EQ}, a hexahistidine-labeled variant with E291Q/E568Q substitutions (glutamine for glutamic acid at positions 291 and 568) in the Walker-B motifs of the D1 and D2 rings to prevent ATP hydrolysis. When His₆-Cdc48^{EQ/EQ} was added to a *T. acidophilum* cell extract, it pulled down two proteins (Fig. 1C), identified as the 20S α and β subunits (fig. S2). Using purified His₆-tagged 20S and purified untagged Cdc48^{EQ/EQ}, we also observed a direct and approximately stoichiometric interaction in the presence of ATP but not adenosine diphosphate (ADP) (Fig. 1E).

The N termini of the α subunits gate or restrict entry of peptides longer than five to seven residues into free 20S, and such peptides are cleaved at enhanced rates by PAN•20S or the 26S proteasome (10). With ATP present, Cdc48 also had gate-opening activity, stimulating 20S cleavage of a nonapeptide but not a tetrapeptide (Fig. 2A and fig. S3). When the gate residues were deleted (10), 20S ^{Δ 2-12} alone cleaved the nonapeptide at a rate similar to that of Cdc48•20S, and added Cdc48 did not increase 20S ^{Δ 2-12} activity (Fig. 2B). Cdc48^{AN}, which lacks the N domain, stimulated nonapeptide cleavage in the presence of ATP and two poorly hydrolyzable analogs, ATP γ S (adenosine 5'-[γ -thio]triphosphate) and AMP-PNP (adenosine 5'-[β , γ -imido]triphosphate), whereas these analogs supported very weak gate opening by Cdc48 (Fig. 2B).

Apparent equilibrium dissociation constants of ~160 nM for Cdc48•20S and ~300 nM for PAN•20S were determined by measuring the Cdc48/PAN dependence of 20S nonapeptide cleavage (Fig. 2C and fig. S4). Cdc48^{AN} bound 20S substantially more tightly, with an affinity of ~2 nM (Fig. 2C).

A Cdc48 variant with the Y753A mutation (alanine replacing Tyr⁷⁵³) in the HbYX motif bound 20S and stimulated peptide cleavage (Fig. 2C) but with decreased affinity (~580 nM) and only 14% of the maximum activity of Cdc48 (Fig. 2D). Thus, binding and gate opening are not fully coupled in this mutant. In the binding pocket on 20S α , Lys⁶⁶ forms a salt bridge with the α -carboxylate of PAN (11). Cdc48 or Cdc48^{AN} stimulated nonapeptide cleavage by 20S^{K66A} poorly (Fig. 2, B and C), which suggests that interactions involving the Cdc48 tail and Lys⁶⁶ facilitate gate opening.

T. acidophilum Cdc48 unfolds green fluorescent protein (GFP) bearing the ssrA degron, with unfolding and ATP hydrolysis being stimulated by 120 mM Mg⁺⁺ or N-domain deletion (12). We found that Cdc48•20S completely degraded native GFP-ssrA in the presence of ATP and 120 mM Mg⁺⁺ (Fig. 3A). Cdc48^{AN}•20S also efficiently degraded GFP-ssrA with ATP and 20 mM Mg⁺⁺, but no degradation was observed with ATP γ S, without Cdc48^{AN}, or with proteolytically inactive 20S^{BT1A} (Fig. 3B). Michaelis-Menten analysis of GFP-ssrA degradation by Cdc48•20S showed that the Michaelis constant K_M was ~2 μ M in high and low Mg⁺⁺, whereas the maximum initial velocity of the enzyme-catalyzed reaction, V_{max} , increased from 0.07 to 0.39 min⁻¹ enzyme⁻¹ as Mg⁺⁺ was raised (Fig. 3C and table S2). For Cdc48^{AN}•20S in low Mg⁺⁺, K_M was 0.15 μ M and V_{max} was 3.2 min⁻¹ enzyme⁻¹. Thus, the N domain of Cdc48 represses proteolytic activity both by increasing K_M and decreasing V_{max} . For comparison, PAN•20S degraded GFP-ssrA with a K_M of 0.15 μ M and V_{max} of 0.35 min⁻¹ enzyme⁻¹ (Fig. 3C and table S2).

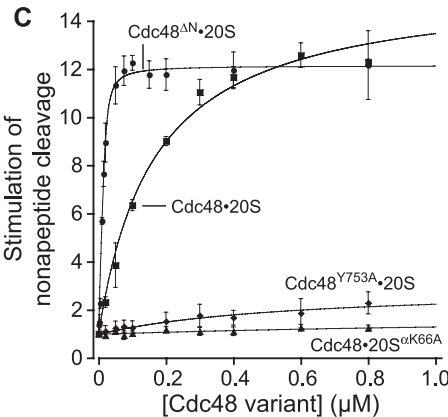
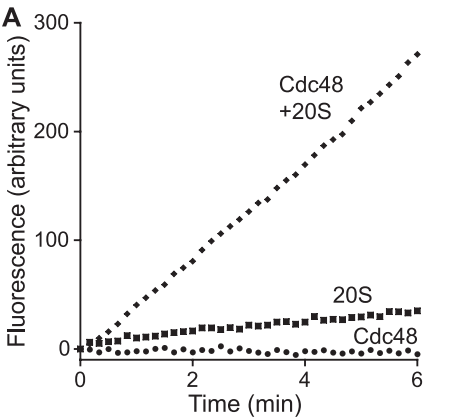
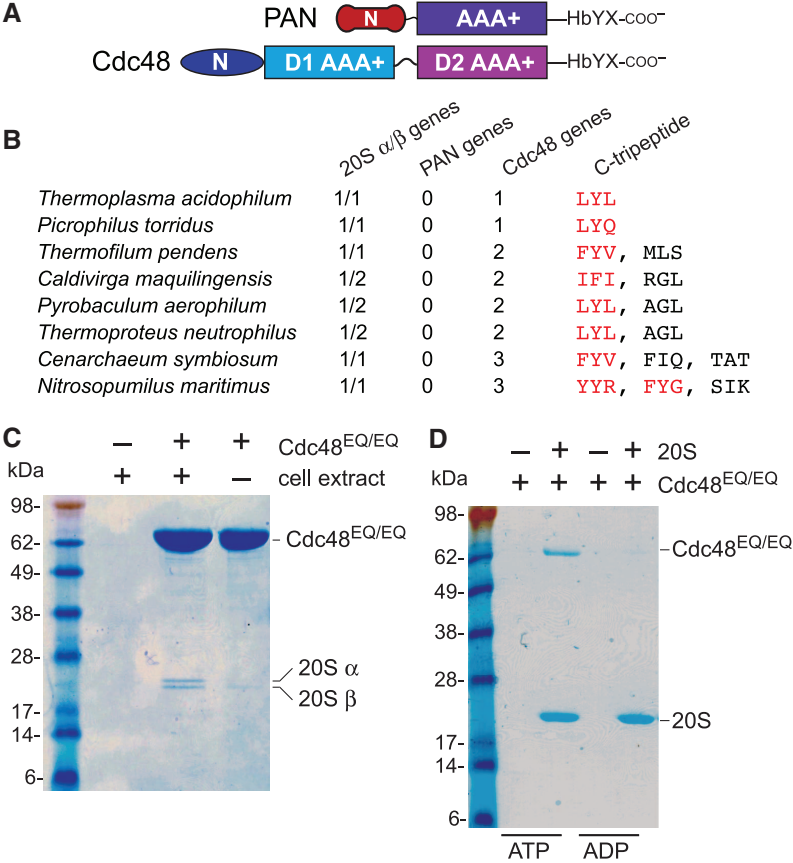
Cdc48•20S degraded CM-titin¹²⁷-ssrA, a variant denatured by carboxymethylation of normally buried cysteines (13), faster than native titin¹²⁷-ssrA (Fig. 3D). Cdc48^{AN}•20S also degraded the denatured substrate more rapidly, in a reaction that required ATP hydrolysis (Fig. 3D). In principle, Cdc48 might unfold and release substrates into solution for subsequent capture and degradation by free 20S. However, degradation of unfolded CM-titin¹²⁷-ssrA by 20S alone was much slower than degradation with Cdc48 or Cdc48^{AN} (Fig. 3D), which suggested that ATP-fueled translocation from Cdc48 into the associated 20S peptidase is responsible for most degradation. Moreover, a PAN variant that unfolded GFP-ssrA, but did not bind 20S, failed to support degradation (figs. S4 and S5). Thus, folded and unfolded substrates are only degraded efficiently by complexes of 20S with Cdc48 or PAN.

A Δ HbYX Cdc48^{AN} variant that terminates with Asp-Gln-Gly supported a low level of gate opening (Fig. 4A) and 20S degradation of GFP-ssrA

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Fig. 1. Cdc48 binds the 20S peptidase. **(A)** PAN and Cdc48 have distinct N domains but homologous AAA+ modules and HbYX motifs. **(B)** Examples of archaea in which PAN was absent but 20S and Cdc48 were present. Red Cdc48 tripeptides match HbYX or related sequences. **(C)** Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) showed that purified *T. acidophilum* His₆-Cdc48^{EQ/EQ} pulled down two proteins from a *T. acidophilum* cell extract, which were identified as the 20S α and β subunits (fig. S2). **(D)** Using purified *T. acidophilum* proteins, 20S with a His₆-tagged β subunit pulled down a roughly stoichiometric quantity of untagged Cdc48^{EQ/EQ} in the presence of 2 mM ATP but not ADP.



D

	maximum fold 20S stimulation	K_{app} 20S dissociation (nM)
Cdc48	14.0 $\pm$ 0.5	160 $\pm$ 16
Cdc48 ^{ΔN}	12.0 $\pm$ 0.2	2.4 $\pm$ 0.6
Cdc48 ^{Y753A}	1.9 $\pm$ 0.8	580 $\pm$ 24
PAN	6.3 $\pm$ 0.3	300 $\pm$ 17

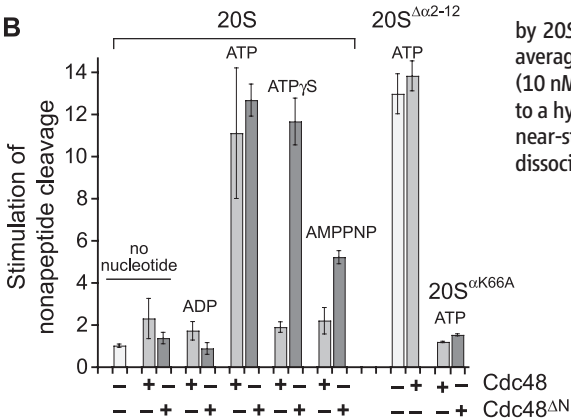


Fig. 2. Cdc48 activates 20S gate opening. **(A)** *T. acidophilum* Cdc48 (0.4 μ M) and 20S (10 nM) cleaved a nonapeptide (10 μ M) rapidly as assayed by increased fluorescence with 2 mM ATP. Cleavage by Cdc48 alone was undetected and by 20S alone was slow. **(B)** Cdc48 or Cdc48^{ΔN} (0.4 μ M) stimulation of nonapeptide cleavage by 20S, 20S^{Δα2-12}, or 20S^{αK66A} (10 nM) in the presence of different nucleotides (2 mM). Values are averages $\pm$ SD ($W \geq 3$) divided by the 20S cleavage rate. **(C)** Nonapeptide cleavage by 20S or 20S^{αK66A} (10 nM) as a function of increasing concentrations of Cdc48 or variants. Solid lines ($R^2 \geq 0.98$) are fits to a hyperbolic equation for all curves except Cdc48^{ΔN}•20S, which was fit to a quadratic equation for near-stoichiometric binding. **(D)** Values for the maximum stimulation of cleavage and the apparent dissociation constant (K_{app}) were calculated from the fits in (C) or fig. S4.

Fig. 3. Protein degradation by the Cdc48•20S proteasome. **(A)** SDS-PAGE assay of GFP-ssrA (10 μ M) degradation by Cdc48 (1 μ M) and 20S (2 μ M) with 2 mM ATP, 120 mM MgCl_2 , and an ATP regeneration system. **(B)** (Top) SDS-PAGE assay of GFP-ssrA (5 μ M) degradation by 20S (0.9 μ M) and Cdc48 $^{\Delta\text{N}}$ (0.3 μ M), 2 mM ATP, 20 mM MgCl_2 , and a regeneration system. (Bottom strips) GFP-ssrA in otherwise comparable assays using 2 mM ATP γ S (no regeneration), without Cdc48 $^{\Delta\text{N}}$, or with catalytically inactive 20S $^{\text{BT1A}}$. **(C)** Michaelis-Menten plots of GFP-ssrA degradation by 20S (0.9 μ M) and Cdc48 (0.3 μ M; left) or 20S (0.9 μ M) with Cdc48 $^{\Delta\text{N}}$ or PAN (0.3 μ M; right). Values are averages $\pm$ SD ($N \geq 3$). Fitted parameters are listed in table S2. **(D)** Degradation of 10 μ M native titin $^{\text{I27}}$ -ssrA or denatured CM-titin $^{\text{I27}}$ -ssrA. The top two gel strips show degradation by Cdc48 (1.2 μ M) and 20S (0.4 μ M) in 120 mM Mg^{++} buffer. The next four strips show degradation by Cdc48 $^{\Delta\text{N}}$ or Cdc48 $^{\Delta\text{N}/\text{EQ}/\text{EQ}}$ (1.2 μ M) and 20S (0.4 μ M) in 20 mM Mg^{++} buffer. The bottom strip shows degradation by 20S (0.4 μ M) in 20 mM Mg^{++} buffer. Reactions contained 5 mM ATP and a regeneration system or 5 mM ATP γ S without regeneration.

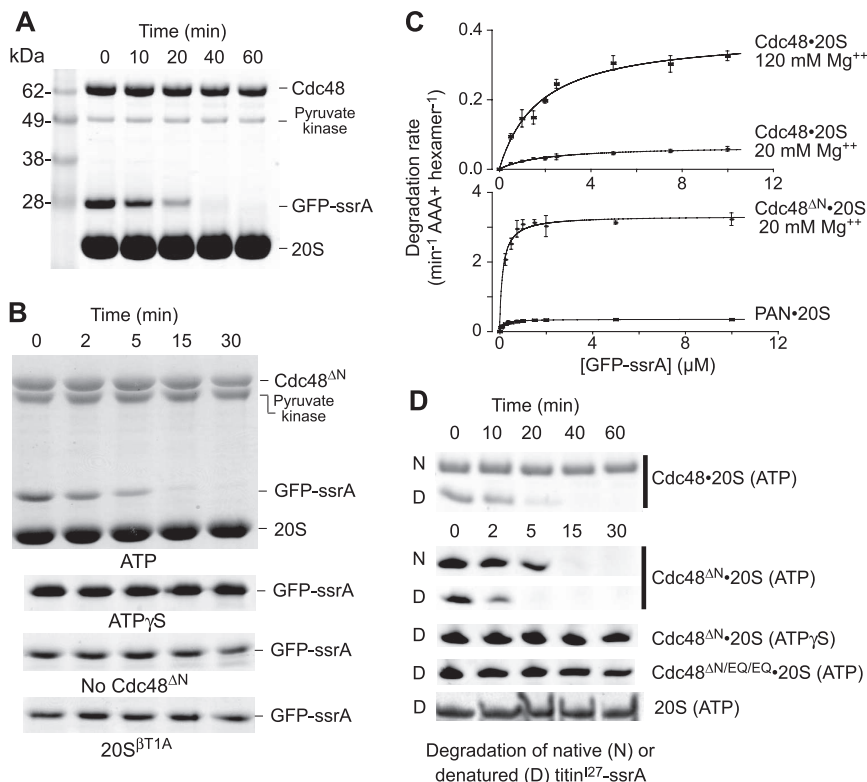
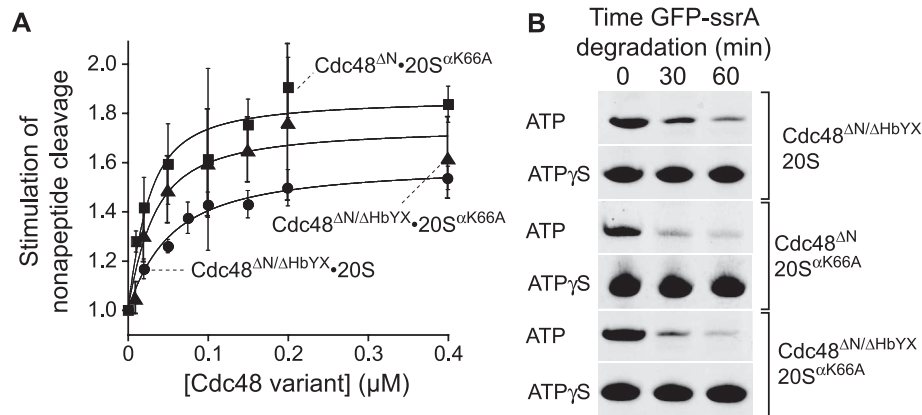


Fig. 4. The HbYX motif is not required for 20S binding and degradation. **(A)** Stimulation of peptide cleavage by 20S or 20S $^{\alpha\text{K66A}}$ (10 nM) as a function of Cdc48 $^{\Delta\text{N}/\text{HbYX}}$ or Cdc48 $^{\Delta\text{N}}$ concentration. Values are averages ($N = 3$) $\pm$ SD. Solid lines ($R^2 \geq 0.948$) are fits to a quadratic equation for near-stoichiometric binding, yielding K_{app} values of 41 ± 4 nM for Cdc48 $^{\Delta\text{N}/\text{HbYX}}$ •20S, 21 ± 9 nM for Cdc48 $^{\Delta\text{N}}$ •20S $^{\alpha\text{K66A}}$, and 15 ± 6 nM for Cdc48 $^{\Delta\text{N}/\text{HbYX}}$ •20S $^{\alpha\text{K66A}}$. **(B)** Gel strips showing ATP-dependent degradation of GFP-ssrA (5 μ M) by Cdc48 $^{\Delta\text{N}/\text{HbYX}}$ •20S, Cdc48 $^{\Delta\text{N}}$ •20S $^{\alpha\text{K66A}}$, or Cdc48 $^{\Delta\text{N}/\text{HbYX}}$ •20S $^{\alpha\text{K66A}}$. Assays were performed in 20 mM Mg^{++} using 5 mM ATP and regeneration or 5 mM ATP γ S; 20S/20S $^{\alpha\text{K66A}}$ (0.4 μ M); Cdc48 $^{\Delta\text{N}}$ /Cdc48 $^{\Delta\text{N}/\text{HbYX}}$ (1.2 μ M).



(Fig. 4B). Although the affinity of Cdc48 $^{\Delta\text{N}/\text{HbYX}}$ for 20S was reduced compared with that of Cdc48 $^{\Delta\text{N}}$, it was still surprisingly strong (40 nM). Because the HbYX motif stabilizes but is not required for 20S binding, other Cdc48 regions must make major contributions to binding. We also observed weak gate opening (Fig. 4A) and GFP-ssrA degradation (Fig. 4B) when we used 20S $^{\alpha\text{K66A}}$ in combination with either Cdc48 $^{\Delta\text{N}}$ or Cdc48 $^{\Delta\text{N}/\text{HbYX}}$, showing that K66 recognition of an α -carboxylate is not essential for binding and degradation. Moreover, the affinity effects of the Cdc48 ΔHbYX and 20S αK66A mutations were nonadditive, as expected for interacting groups. How can the protein degradation activities of Cdc48 $^{\Delta\text{N}/\text{HbYX}}$ •20S, Cdc48 $^{\Delta\text{N}}$ •20S $^{\alpha\text{K66A}}$, and Cdc48 $^{\Delta\text{N}/\text{HbYX}}$ •20S $^{\alpha\text{K66A}}$, which require an open 20S gate, be reconciled with

the poor stimulation of peptide cleavage by these enzymes? The 20S gate dynamically adopts a variety of conformations (14), and an actively translocating polypeptide might trap a transiently open gate with the spooling chain preventing closure.

We find that archaeal Cdc48 binds to the proteasomal 20S peptidase, induces gate opening, and unfolds and actively translocates protein substrates into the 20S chamber for proteolysis. Cdc48 is the only 20S partner in ~15% of archaea, and Cdc48 and PAN are both present in the remaining ~85%. On the basis of their C-terminal sequences (table S1), the fact that Cdc48 binding is not strictly HbYX-dependent, and our finding that Cdc48 and PAN bind 20S with similar affinities (Fig. 2D), it appears that either en-

zyme could combine with 20S to form alternative proteasomes in most archaea. The N domain of Cdc48 is a tethering site for adaptors and possibly substrates, and there is evidence that it controls hexamer conformation and ATP-hydrolysis rates (12, 15–17). In our experiments, deleting the N domain of *T. acidophilum* Cdc48 strengthened 20S binding and increased proteolytic activity. Thus, N-domain modification or N-domain binding of substrates and adaptors may regulate intracellular degradation by the Cdc48•20S proteasome.

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The data reported in this manuscript are presented in the main paper and supplementary materials.

Supplemental Materials

www.sciencemag.org/cgi/content/full/science.1224352/DC1
Materials and Methods
Figs. S1 to S5
Tables S1 and S2
References (18–23)

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The Double-Edged Sword: Does Biomechanism Increase or Decrease Judges' Sentencing of Psychopaths?

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We tested whether expert testimony concerning a biomechanism of psychopathy increases or decreases punishment. In a nationwide experiment, U.S. state trial judges ($N = 181$) read a hypothetical case (based on an actual case) where the convict was diagnosed with psychopathy. Evidence presented at sentencing in support of a biomechanical cause of the convict's psychopathy significantly reduced the extent to which psychopathy was rated as aggravating and significantly reduced sentencing (from 13.93 years to 12.83 years). Content analysis of judges' reasoning indicated that even though the majority of judges listed aggravating factors (86.7%), the biomechanical evidence increased the proportion of judges listing mitigating factors (from 29.7 to 47.8%). Our results contribute to the literature on how biological explanations of behavior figure into theories of culpability and punishment.

Scientists have identified associations between genetic, neurobiological, and environmental factors and antisocial behaviors such as psychopathy [see, for example, (1–5)]. The inferences drawn from these associations are varied and controversial. But controversy has not prevented the research from making its way into court. Forensic psychiatrists reported testing defendants charged with first-degree murder for their genetic monoamine oxidase-A (MAOA) status (6), and an Italian judge considered a defendant's MAOA status when rendering his sentence (7). Neuroscientists performed functional magnetic resonance imaging on convicted murderer Brian Dugan to determine whether he has a defective, psychopathic brain, as ostensibly evidenced by neuroimaging (8).

Courts have repeatedly held that evidence relating to both future dangerousness and impaired volitional control is highly relevant to sentencing decisions (9, 10). Because of this, commentators have pointed out that evidence of a biomechanism associated with antisocial behaviors such as psychopathy may present a double-edged sword

(11–18). And, indeed, some judges have considered such evidence mitigating (i.e., decreasing sentence), whereas other judges have considered the evidence aggravating (i.e., increasing sentence) (7, 19). Both sides of the double-edged sword rely in part on hard biological determinism, or the idea that once we know something about an individual's genes or brain, we can predict or explain his or her behavior (20). Thus, under a retributive or “just desserts” theory of punishment, evidence of a biomechanism may reduce judgments of culpability because it identifies an internal and stable cause of behavior believed to be outside the individual's control (21–23). At the same time, under a utilitarian theory that seeks to promote social welfare, the biomechanism may increase punishment, as the immutable characteristic suggests that he or she will likely reoffend. Such determinist perceptions of immutability may be especially pronounced for biomechanisms involving DNA-based evidence (24, 25). Past research has confirmed that judges are willing to hear and weigh evidence concerning genetic risk factors in criminal cases (26). However, the effect of biomechanical evidence on judges' reasoning in sentencing has yet to be experimentally tested.

Participants were 181 U.S. state trial court judges recruited by e-mail to complete an anonymous online survey concerning judicial use of

scientific evidence at sentencing. All judges were presented with the same hypothetical vignette (~1200 words) loosely based upon the actual case of *Mobley v. State* (27), which involved a convicted murderer who famously sought to be genetically tested for his MAOA status as part of his defense. In our vignette, Jonathan Donahue attempted to rob a restaurant. When the manager did not give Donahue the money, Donahue struck him repeatedly in the head with a gun, resulting in moderate, permanent brain damage. A jury found Donahue guilty of aggravated battery (see supplementary materials for recruitment procedure, judges' demographic information, a discussion of potential selection bias, and experimental vignette).

Participants were randomly assigned to one cell of a 2 “presenting party” (prosecution/defense) $\times$ 2 “biomechanism” (absent/present) design. All participants were provided identical expert testimony from a psychiatrist explaining that Donahue was a diagnosed psychopath. Participants in the biomechanism-absent condition received only expert testimony concerning the diagnosis of psychopathy. Participants in the biomechanism-present condition received identical expert testimony concerning the diagnosis of psychopathy plus expert testimony from a neurobiologist who presented an explanation of the biomechanism contributing to the development of psychopathy (here, low MAOA activity, atypical amygdala function, and other neurodevelopmental factors) (28). In the prosecution condition, prosecutors argued that the evidence should be considered aggravating because the crime and Donahue's actions afterward all pointed to his being a psychopath and posing a continued threat to society. In the defense condition, the defense counsel argued that the evidence should be considered mitigating because the crime and his actions afterward all pointed to Donahue's having a harder time controlling his impulses due to his disorder (see supplementary materials for complete text of expert testimony).

After exposure to the full case, including all expert testimony, judges were asked to indicate the extent to which the evidence concerning psychopathy mitigated, aggravated, or had no effect on the punishment they would render to Donahue; to rate his legal responsibility, moral responsibility, and free will; to estimate their per-

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sonal average sentence for aggravated battery; and finally, to provide their sentence in years for the present case. Following each question, judges were asked to explain their answers using an open-ended text box (see supplementary materials for complete measures and examples of judges' answers).

The evidence concerning psychopathy was rated as aggravating overall ($M = 3.59$, $SEM = 0.068$). A two-way analysis of variance (ANOVA) yielded significant main effects for both biomechanism ($F_{1,176} = 10.411$, $P < 0.001$) and presenting party ($F_{1,176} = 13.482$, $P < 0.0001$). As shown in Fig. 1, the expert testimony concerning a biomechanism significantly reduced judgments of aggravation (biomechanism present: $M = 3.37$, $SEM = 0.099$ versus biomechanism absent: $M = 3.80$, $SEM = 0.087$). Expert testimony about psychopathy presented by the defense also reduced judgments of aggravation ($M = 3.31$, $SEM = 0.108$), compared with identical psychopathy information presented by the prosecution ($M = 3.80$, $SEM = 0.080$). The two-way interaction was not significant ($F_{1,176} = 0.368$, $P < 0.545$).

Complete sentencing data were provided by 164 respondents (90.6% of judges). The average sentence was 12.93 years and highly variable ($SEM = 0.682$; range = 1 to 41), with significant variation by state of adjudication ($F_{18,145} = 5.068$, $P < 0.0001$). This is not surprising, as no two states have exactly the same sentencing guidelines; they differ in factors such as evidentiary rules, the role of parole boards, the impact of the presence of a firearm, and the degree to which judges may exercise discretion. To include state of adjudication as a factor, we collapsed across presenting party (which had no significant main effects or interactions on sentencing; see supplementary materials). The resulting state of adjudication $\times$ biomechanism ANOVA yielded main effects of state of adjudication, $F_{10,113} = 8.340$, $P < 0.0001$, and biomechanism, $F_{1,113} = 5.372$, $P < 0.022$. The presentation of a biomechanism resulted in lower sentences overall (biomechanism present: $M = 12.83$ years, $SEM = 1.104$, $n = 62$ versus biomechanism absent: $M = 13.93$ years, $SEM = 1.039$, $n = 73$). There were no other reliable effects (see supplementary materials for detailed information about state differences in sentencing and alternative methods for accounting for the variability among states). Finally, sentences in both conditions significantly exceeded the judges' reported personal averages for aggravated battery (biomechanism present: $M = 9.040$, $SEM = 0.808$, $t(61) = 6.148$, $P < 0.0001$; biomechanism absent: $M = 9.336$, $SEM = 0.731$, $t(72) = 7.825$, $P < 0.0001$). Thus, despite the significant variability among states when it came to sentencing, the addition of a biomechanism for psychopathy significantly reduced the sentence and significantly reduced the degree to which psychopathy was rated as aggravating. The idea that identifying a biomechanical cause by itself excuses behavior has been called the "psycho-legal error," because all behavior is ul-

timately caused and so all behavior could, in turn, be excused (25). The biomechanical cause is only relevant to legal evaluations of behavior if it is tied to some normative theory about how the biomechanical cause actually compromises legal responsibility. With that in mind, we also asked the judges to rate Donahue's free will, legal responsibility, and moral responsibility.

There were no experimental effects concerning biomechanism or presenting party (all F s < 1 , not significant) for questions concerning legal responsibility ($M = 4.90$, $SEM = 0.035$; $n = 174$), moral responsibility ($M = 4.27$, $SEM = 0.088$; $n = 168$), or free will ($M = 4.66$, $SEM = 0.049$; $n = 169$), as these judgments were uniformly high and, in the case of legal responsibility and free will, near the scale's maximum. This disconnect between the significant effects for reduced aggravation and reduced sentencing, but lack of corresponding effects for reduced free will and responsibility, presents an apparent paradox, as free will, moral responsibility, and punishment are often taken to be closely connected (29). One could discount the relevance of philosophical musings on free will and responsibility when it comes to actual judicial reasoning (30), and indeed, some judges responded to the questions about free will and moral responsibility with answers such as, "It is not my job to judge morals. It is my job to judge legal issues." (See the next section and supplementary materials for additional samples of judges' reasoning.) But such discounting then requires an alternative explanation for the effects of reduced aggravation and reduced sentence. Although these results await replication in less extreme cases, with more nuanced means of evaluating relative degrees of constraint on defendant's free will and responsibility, they should be considered in conjunction with the following analysis of judges' stated reasons for their sentencing decisions.

Judges sometimes issue opinions that explain the theoretical basis for their sentences.

But usually we are left guessing about how specific theories of punishment compete with one another in the judges' minds. In our experiment, we were able to both capture and analyze judges' reasons in light of competing punishment theories. Judges' explanations were coded by two independent raters with high agreement (see Table 1 for kappa statistics and coding categories).

As shown in Table 1 and Fig. 2, consistent with the highly aggravating nature of the crime, the vast majority of judges (86.7% overall) listed at least one aggravating factor when reasoning through their responses, with explanations concerning the convict's future dangerousness, inability to change, control over and intentionality of his actions most frequently listed. As shown in Fig. 2, although the introduction of a biomechanism for psychopathy tended to increase the percentage of judges listing at least one aggravating factor, this result was only marginally significant (from 82.4 to 91.1%, $\chi^2(1) = 2.973$, $P < 0.085$).

Although mitigating factors were mentioned less frequently (38.7% of judges overall), the presentation of a biomechanism by the defense doubled the number of judges listing a mitigating factor (65.9% versus 27.8 to 32.7%; $\chi^2(3) = 16.830$, $P < 0.001$). As shown in Table 1, the most frequently listed mitigating factors were that the convict was mentally ill and lacked control over his actions, and thus was less legally culpable. Judges justified these responses with comments such as, "The evidence that psychopaths do not have the necessary neural connections to feel empathy is significant. It makes possible an argument that psychopaths are, in a sense, morally 'disabled' just as other people are physically disabled. I have received and considered such evidence in past trials."

Finally, as shown at the bottom of Table 1, we coded explicit mentions of the concepts of balancing and weighing both mitigating and aggravating factors, which were derived from statements independently coded as either mitigating

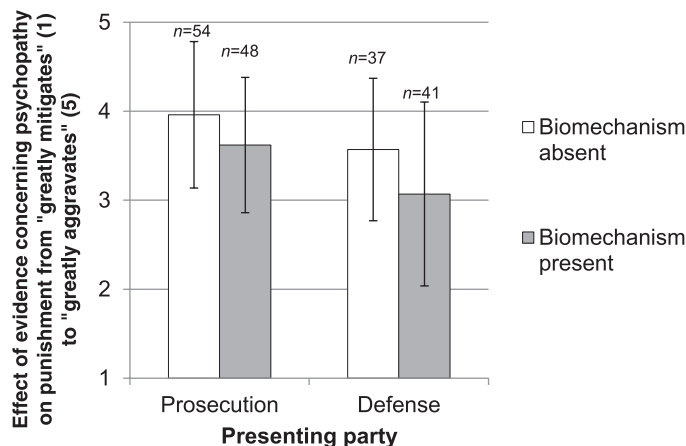


Fig. 1. Judgments of the mitigating versus aggravating effects of evidence concerning psychopathy on punishment as a function of presenting party and the presentation of a biomechanism. Error bars represent ± 1 SD.

Table 1. Number and proportion of judges citing any aggravating or mitigating factor and specific aggravating or mitigating factors as a function of the presentation of a biomechanism and presenting party in response to any of the ques-

tions asking them to explain their reasoning about the effects of expert testimony concerning psychopathy on judgment, along with description of each content code and its interrater reliability (34). Number in parentheses shows the proportion.

		Biomechanism				Total (N = 181)
Kappa		Absent		Present		
		Prosecution (n = 54)	Defense (n = 37)	Prosecution (n = 49)	Defense (n = 41)	
<i>Aggravating factors</i>						
Any aggravating factor		45 (0.833)	30 (0.811)	45 (0.918)	37 (0.902)	157 (0.867)
Aggravates (increases sentence)	1.00	26 (0.481)	14 (0.378)	16 (0.327)	12 (0.293)	68 (0.376)
Future dangerousness and incapacitation	1.00	27 (0.500)	19 (0.514)	32 (0.653)	22 (0.537)	100 (0.553)
Won't change (recidivism)	1.00	21 (0.389)	18 (0.487)	19 (0.388)	14 (0.341)	72 (0.398)
Seriousness of the crime and nature of the harm to victim	0.987	24 (0.444)	9 (0.243)	14 (0.286)	15 (0.366)	62 (0.343)
Lack of remorse/guilt	1.00	11 (0.204)	4 (0.108)	8 (0.163)	10 (0.244)	33 (0.182)
Lack of empathy (cold-blooded)	1.00	1 (0.019)	1 (0.027)	5 (0.102)	5 (0.122)	12 (0.066)
Had control	1.00	20 (0.370)	9 (0.243)	25 (0.510)	22 (0.537)	76 (0.420)
Culpable (deserves punishment)	1.00	11 (0.204)	8 (0.216)	14 (0.286)	10 (0.244)	43 (0.238)
Guilty mind (appreciates wrongfulness of actions)	1.00	20 (0.370)	18 (0.487)	19 (0.388)	21 (0.512)	78 (0.431)
Not mentally ill	0.982	11 (0.204)	6 (0.162)	18 (0.367)	7 (0.171)	42 (0.232)
<i>Mitigating factors</i>						
Any mitigating factor		15 (0.278)	12 (0.324)	16 (0.327)	27 (0.659)	70 (0.387)
Mitigates (reduces sentence)	1.00	2 (0.037)	5 (0.135)	2 (0.041)	8 (0.195)	17 (0.094)
Lacks control	1.00	5 (0.093)	4 (0.108)	6 (0.122)	11 (0.268)	26 (0.144)
Mentally ill	1.00	8 (0.148)	5 (0.135)	8 (0.163)	11 (0.268)	32 (0.177)
Not culpable	1.00	4 (0.074)	5 (0.135)	4 (0.082)	5 (0.122)	18 (0.099)
Feel sorry (for defendant)	1.00	0	0	1 (0.020)	2 (0.049)	3 (0.017)
Lacks empathy (defendant is incapable of feeling sorry)	1.00	2 (0.037)	1 (0.027)	4 (0.082)	4 (0.098)	11 (0.061)
<i>Double-edged sword</i>						
Balance/weigh	1.00	10 (0.185)	5 (0.135)	9 (0.184)	19 (0.463)	43 (0.238)

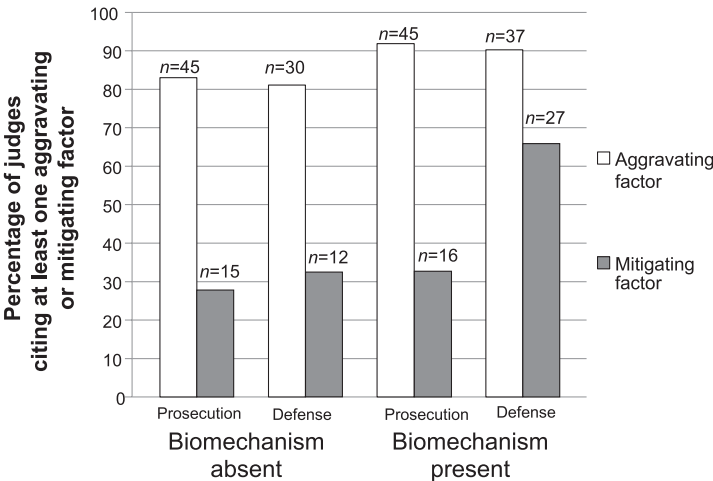


Fig. 2. Percentage of judges citing at least one aggravating factor and at least one mitigating factor, respectively, when asked to explain their reasoning about the impact of the evidence concerning psychopathy on their judgment of the defendant as a function of presenting party and the presentation of a biomechanism. *N*s indicate the number of judges per experimental condition who mentioned an aggravating or mitigating factor, respectively.

or aggravating. Mentions of balancing or weighing these countervailing factors were ~2.5 times as high in the defense/biomechanism-present condition (46.3% versus 13.5 to 18.5% in the other three conditions, $\chi^2(3) = 15.293$, $P < 0.002$). Judges justified these responses with comments such as, “Psychopathy may make the defendant less morally culpable, but it increases his future

dangerousness to society. In my mind, these factors balance out...” These uncommon qualitative data illustrate that the introduction of expert testimony concerning a biomechanism for psychopathy significantly increased the number of judges invoking mitigating factors in their reasoning and balancing them with aggravating factors. These findings

suggest that the biomechanism did invoke such concepts as reduced culpability due to lack of impulse control, even if these concepts did not affect the ratings of free will and responsibility. In an actual case, judges may acknowledge (but not necessarily endorse) the potential for evidence to be seen as mitigating to document their consideration of this evidence in the event of an appeal. However, the demand to do so here in this hypothetical case should have been minimized, and the increased mention of mitigating factors is consistent with the results obtained for reduced ratings of aggravation and reduced sentence. The fact that the increased mention of mitigating factors occurred in conjunction with increased mentions of balancing and/or weighing the aggravating and mitigating factors conveys the genuine double-edged nature of the evidence of a biomechanism. The qualitative data concerning balancing and weighing countervailing factors illustrate how judges may acknowledge a punishment theory, such as retributivism, and find reduced impulse control to be a valid mitigating factor, while simultaneously recognizing the need to weigh different policy goals (e.g., public safety) along the lines of a more utilitarian theory of incapacitation. Our study is both hindered and helped by the extreme facts of our case. The crime itself was violent and reprehensible. Moreover, the convict was portrayed in an unsympathetic manner, bragging about his crime and expressing no remorse.

On the other hand, had the convict been found guilty of murder and had thus faced either the death sentence or life in prison without the possibility of parole, then future dangerousness would have lost its appeal as the defendant might have been incarcerated for life with no potential to reoffend. Further, judges were explicitly told that rehabilitation was not an alternative, as large-scale treatment has to date been ineffective for adult psychopaths. This eliminated a possible third option for those seeking to find a compromise between aggravation and mitigation. In future studies, the potential for effective treatment makes presentation of a third rehabilitative theory, with corresponding expert testimony, an attractive option for testing. The judges were also not presented with cross-examination, having only received the expert scientific testimony from either the defense or the prosecution.

Turning to limitations associated with the science, we focused exclusively on psychopathy, a diagnosis with much stigma. Thus, these results may not generalize to other psychiatric diagnoses associated with antisocial behavior (31, 32). Likewise, we based our account of the biomechanism of psychopathy on James Blair's neurocognitive model (28). Changing this causal description might lead to different judgments (33). Finally, we combined psychiatric, genetic, and neurobiological science in constructing the expert testimony. Future research should examine the effects of separately introducing testimony on genetic penetrance and expression, neurodevelopment and plasticity, and probabilistic data indicating how

much of the relative risk of developing the particular antisocial disorder can be attributed to the given biomechanism.

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34. Answers are collapsed across eliciting question (mitigating versus aggravating effect of evidence, legal responsibility, moral responsibility, free will, and sentencing). For verbatim examples of judges' reasoning, see supplementary materials.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/337/6096/846/DC1
Materials and Methods
Supplementary Text
Figs. S1 and S2
Tables S1 to S3
References
Data File S1

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Hippocampal Place Fields Emerge upon Single-Cell Manipulation of Excitability During Behavior

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The origin of the spatial receptive fields of hippocampal place cells has not been established. A hippocampal CA1 pyramidal cell receives thousands of synaptic inputs, mostly from other spatially tuned neurons; however, how the postsynaptic neuron's cellular properties determine the response to these inputs during behavior is unknown. We discovered that, contrary to expectations from basic models of place cells and neuronal integration, a small, spatially uniform depolarization of the spatially untuned somatic membrane potential of a silent cell leads to the sudden and reversible emergence of a spatially tuned subthreshold response and place-field spiking. Such gating of inputs by postsynaptic neuronal excitability reveals a cellular mechanism for receptive field origin and may be critical for the formation of hippocampal memory representations.

The hippocampus plays a crucial role in the formation of long-term memories for facts and events in humans and for spatial learning in rodents (1). When a rodent explores an en-

vironment, spatial information is prominently represented in the spiking activity of a substantial, environment-specific subset of hippocampal pyramidal neurons. Each such "place cell" fires action potentials (APs) selectively whenever the animal is in a particular region—called the cell's place field—within the environment (2), whereas the remaining neurons, called silent cells, fire few or no spikes (3). Similarly, subsets of human hip-

pocampal neurons spike selectively for specific items or episodes over a background of low-firing rate cells (4). The establishment and stability of these stimulus-specific spiking responses are believed to be the neural basis of hippocampal-dependent learning and memory.

What is the origin of the spatially selective firing of place cells? Each pyramidal cell in hippocampal subregion CA1 receives excitatory inputs from other spatially tuned neurons (5, 6). Therefore, models have generally assumed that the critical factor is the environmental location where each input fires, with summation of these inputs (their firing rates times synaptic weights) followed by thresholding leading to various degrees of spatially selective output spiking or silence (7–11). Indeed, intracellular recordings have revealed that place cells have a region with clearly elevated somatic membrane potential (V_m)—i.e., a "hill"—under their place-field spiking (12–14), whereas silent cells have a flat, spatially uniform V_m (14).

However, another critical aspect of neuronal integration is the interaction of synaptic inputs with the membrane properties of the postsynaptic cell. Inputs can be strongly filtered or amplified (15, 16) before determining output spiking, yet such features have not been included in place cell models. With intracellular recording, place cells were, unexpectedly, also found to be more excitable than silent

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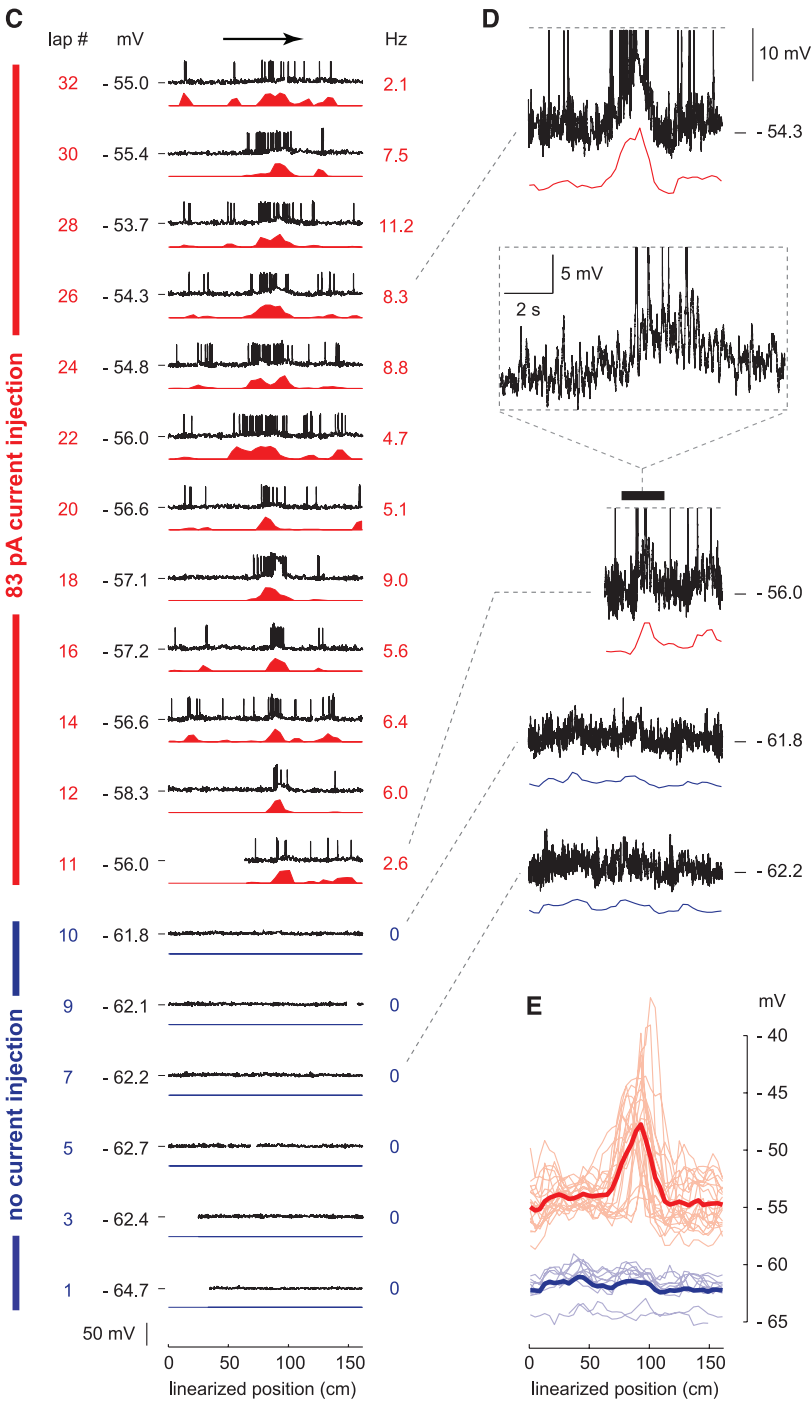
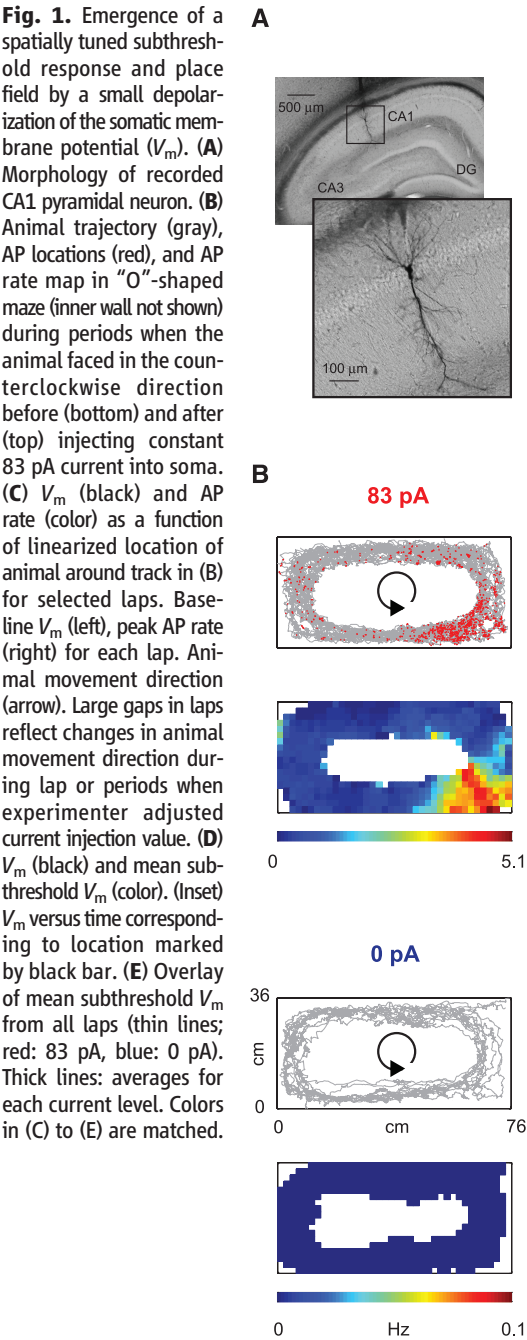
cells—even before the animal encountered the environment (14)—which suggested that cellular properties strongly influence the response to inputs during behavior.

How can one separate the role of cellular properties from inputs in the origin of place cell firing? One approach would be to test whether increasing excitability without altering synaptic inputs can convert a previously spatially untuned silent cell into a place cell. A straightforward way to increase excitability is to depolarize a neuron's baseline V_m by injecting a constant, i.e., spatially uniform, current into the soma and thus bias it toward spiking. Here,

we used the whole-cell recording method to precisely manipulate the somatic V_m in single CA1 pyramidal neurons while rats explored a novel environment, then measured the spatial distribution of subthreshold and spiking responses to see the effect on the integration of inputs.

We recorded 10 silent cells (Fig. 1A) in 10 rats (14, 17, 18) as they moved around an oval track (mean recording duration during behavior was 28 min), in four cases in both directions. Because place cells can have fields in a single direction in such environments, we treated the 14 directions separately. For each direction, animals first explored each

location in the maze at least twice with no applied current (Fig. 1, B and C). The flat mean V_m as a function of location (Fig. 1, C to E) (14) suggests that silent cells receive either few inputs or inputs whose overall tuning is spatially uniform. Alternatively, silent cells may receive substantial spatially tuned input, but their lower excitability severely limits or prevents propagation to the soma. It was surprising that the latter was the case. Depolarization of the somatic baseline V_m by constant current injection immediately caused not only the appearance of a spiking place field (Fig. 1, B and C) but also an underlying subthreshold (i.e., excluding



APs and calcium spikes) V_m hill (Fig. 1, D and E, red), both stable across laps. Of five directions (from five cells) in which a single current level was applied, a single spiking place field and spatially tuned subthreshold hill emerged in two cases (Fig. 1 and fig. S1): multiple, stable hills in one case and firing covering most of the maze in the other cases. This suggested that the number and size of place fields vary over a narrow voltage range.

Therefore, we asked whether, by applying different current levels across laps (five cells, nine directions), we could always create a single, narrow field, as expected in environments of this size. In four of five cells, narrow fields were produced in at least one direction, for a total of six such fields from five directions (one direction had two fields). For population analyses, we included these six fields plus the two from single-current-level experiments. Examples for which, at an intermediate level of depolarization, a single, narrow field was produced (red) are shown in Fig. 2 and fig. S2. Laps were ordered

by baseline V_m because this best predicted resulting subthreshold and spiking activity. With more depolarization, subthreshold and spiking responses broadened (orange). Field emergence depended on baseline V_m , not current injection itself, as illustrated in fig. S3.

For four fields, we returned to the original baseline V_m (by returning to 0 pA) intermittently. In these cases, the field disappeared (Fig. 2A, laps 7 and 12, and fig. S4). Thus, field emergence was not simply experience-dependent. Also, the presence of the field for a few laps was apparently insufficient to induce plasticity that could then maintain the field without depolarization.

Within the (eventual) region of the created field, the amplitude of the hill (“peak – baseline” in Fig. 3A) as a function of baseline V_m showed a sharp rise above a certain baseline V_m value (Fig. 3B). For each field, we estimated the baseline V_m at the midpoint of the rise ($V_{m, \text{gate}} = -56.6 \pm 1.0$ mV) then aligned the fields with respect to $V_{m, \text{gate}}$. The pooled

result (Fig. 3C) showed a thresholdlike transition from a low to maximal amplitude hill within a ~1- to 3-mV range of baseline V_m values, revealing striking sensitivity of a neuron to inputs around its own “threshold” value. This suggests that nonlinear voltage-dependent mechanisms underlie the generation of the subthreshold place field.

We checked several possible sources for the emergence of the hill. First, hills did not require the generation of somatic APs. Laps with baseline V_m within the transition range displayed a clear subthreshold hill without APs (Fig. 3D). Also, the ascending slope of a hill could begin before any spiking in the field in the first lap with current injection (Fig. 1D, inset).

Was there a small, preexisting hill at the eventual location of the field that was amplified by depolarization? No, the mean in-field and out-of-field V_m in the initial 0 pA laps did not differ across the population (difference = 0.03 ± 0.05 mV, $P = 0.49$) (Fig. 3E) or for individual fields (fig. S5).

Alternatively, the field could emerge from a location with no difference in mean V_m but an elevated input resistance (R_N) (due to decreased excitatory and inhibitory input), which would depolarize more than regions with lower R_N in response to a constant current. In this case, the initial appearance of the hill could be independent of voltage-gated mechanisms. However, for two of the three fields we could test, there was no evidence of this ($P = 0.24$, $P = 0.47$).

We then checked whether the subthreshold response emerged from regions with larger fluctuations in V_m (but no difference in mean V_m). Neither the standard deviation ($P = 0.25$) (Fig. 3E), nor gamma (25 to 100 Hz) band power ($P = 0.63$), nor right tail of the distribution (fig. S6) of V_m were initially different inside versus outside the eventual field location. We examined the prominent theta (4 to 10 Hz) band in more detail. Theta power was higher within the field for laps with fields ($P = 0.01$) (Fig. 4, A and B) (13), but, again, not in the initial 0 pA laps ($P = 0.89$) (fig. S7 and Fig. 4B). Rather, theta power varied with V_m and not location per se (i.e., the power- V_m relation did not differ inside and outside the field) (Fig. 4C and fig. S8).

Therefore, there was no obvious indication of where the field would eventually emerge. When it did emerge, the subthreshold response could remain elevated for an extended period (4.8 ± 0.5 s) while the animal remained in the field (Fig. 1D, inset, and Fig. 4A).

In two recordings of place, as opposed to silent cells, we suppressed spiking with hyperpolarizing current. In one case, the original subthreshold hill disappeared (fig. S9), which provides further evidence that place fields may not originate from passive summation of synaptic input in novel environments.

Although any spatial information in the hippocampus must ultimately come from external inputs (5, 19–21), these findings directly demonstrate that nonspatial, cellular factors can play a decisive role in how neurons respond to inputs during behavior. In particular, most CA1 pyramidal cells receive spatially tuned synaptic input that, in silent cells, does

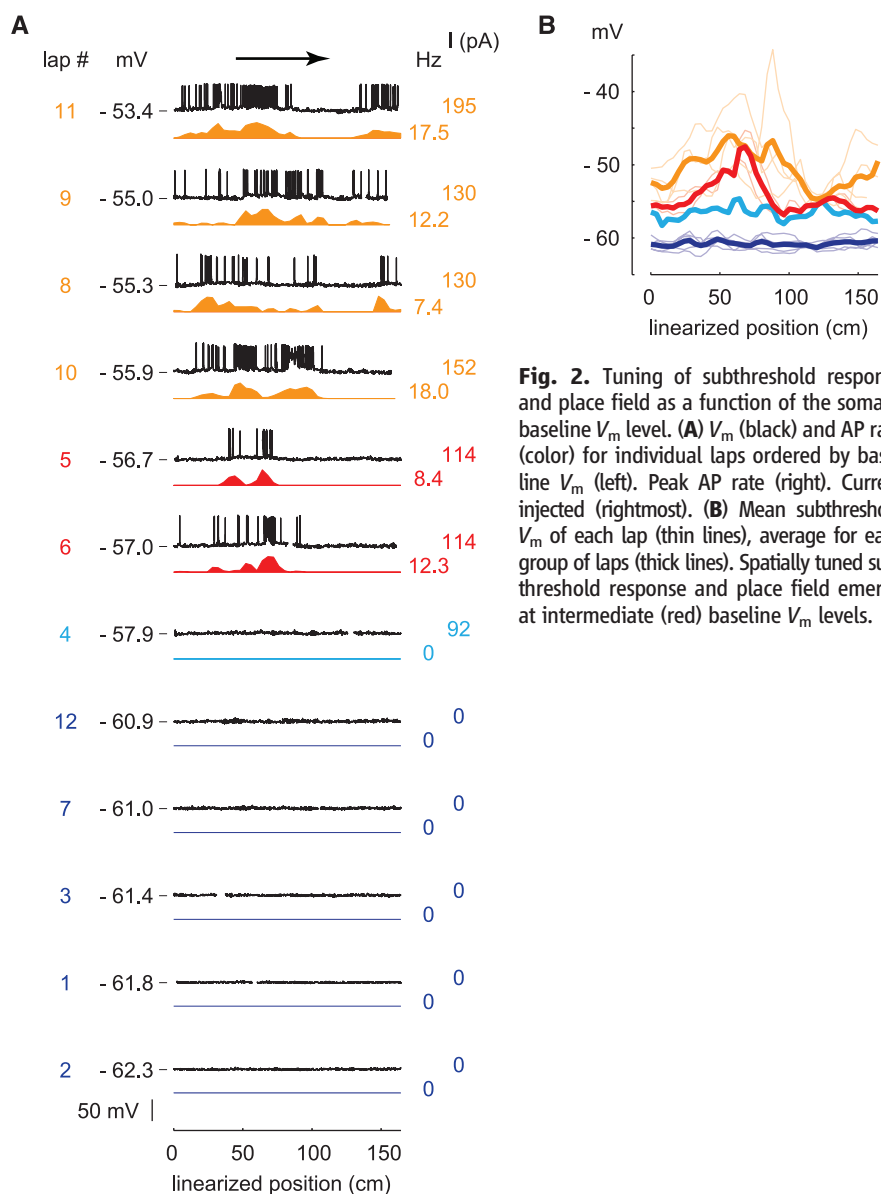


Fig. 2. Tuning of subthreshold response and place field as a function of the somatic baseline V_m level. (A) V_m (black) and AP rate (color) for individual laps ordered by baseline V_m (left). Peak AP rate (right). Current injected (rightmost). (B) Mean subthreshold V_m of each lap (thin lines), average for each group of laps (thick lines). Spatially tuned subthreshold response and place field emerge at intermediate (red) baseline V_m levels.

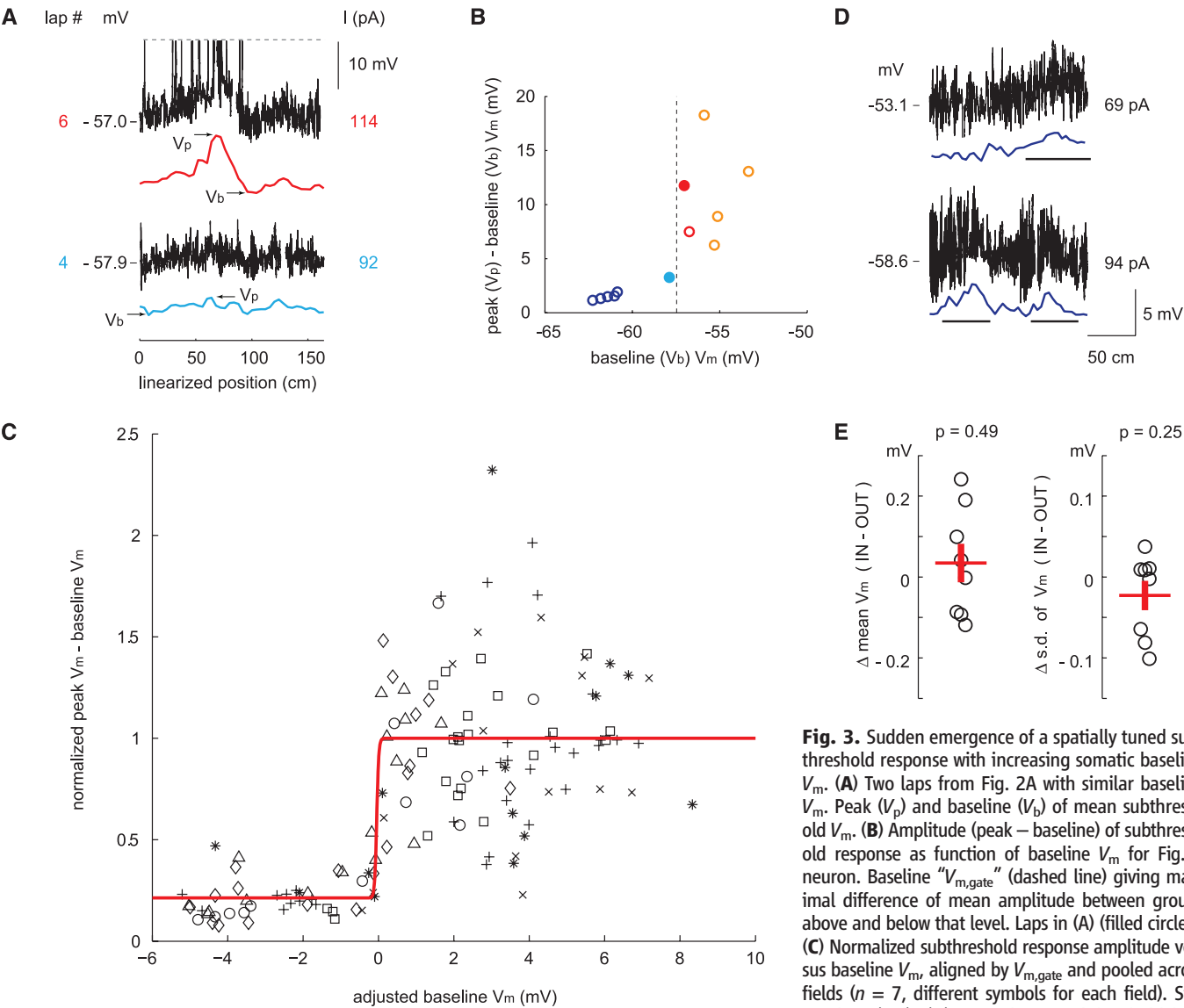
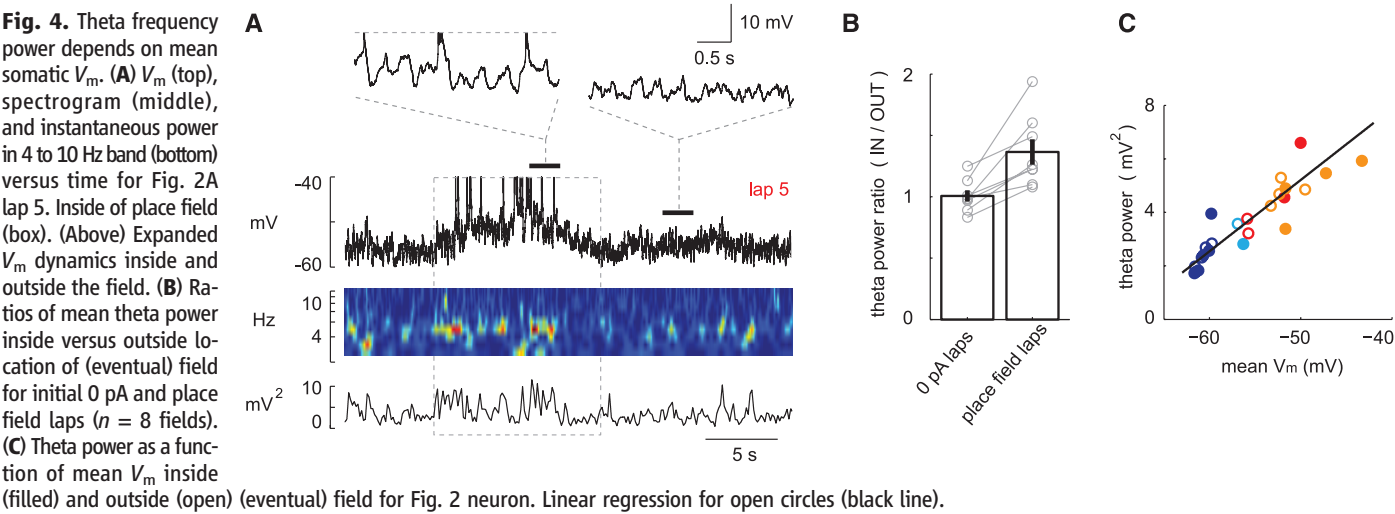


Fig. 3. Sudden emergence of a spatially tuned subthreshold response with increasing somatic baseline V_m . **(A)** Two laps from Fig. 2A with similar baseline V_m . Peak (V_p) and baseline (V_b) of mean subthreshold V_m . **(B)** Amplitude (peak – baseline) of subthreshold response as function of baseline V_m for Fig. 2 neuron. Baseline “ $V_{m, gate}$ ” (dashed line) giving maximal difference of mean amplitude between groups above and below that level. Laps in **(A)** (filled circles). **(C)** Normalized subthreshold response amplitude versus baseline V_m , aligned by $V_{m, gate}$ and pooled across fields ($n = 7$, different symbols for each field). Sigmoid fit (red). **(D)** Examples of subthreshold hills

without APs (horizontal bars: eventual field locations). **(E)** Difference between mean and standard deviation of V_m inside and outside location of eventual field in initial 0 pA laps ($n = 8$ fields).



not propagate to the soma to yield place-field activity. However, a spatially uniform signal that slightly depolarizes the soma can reveal this input. This gating of inputs, as opposed to just outputs, by somatic V_m constitutes a novel mechanism for receptive fields. It also implies that integration of multiple inputs is gated by somatic V_m (fig. S10).

These results are the opposite of those expected from standard models of cortical receptive fields (22, 23) and place cells (7–11). In these models, a neuron receives more excitatory synaptic input in response to the preferred stimulus, and this input is passively summed then compared with the AP threshold. Silent cells would have spatially tuned somatic V_m hills of various amplitudes that do not reach threshold, and somatic depolarization would reduce the driving force and, thus, hill amplitude (23).

At the other extreme is a model in which synaptic inputs are uniformly distributed in stimulus space, and the neuron selectively amplifies a subset of them. This possibility is supported by recent *in vivo* work in visual cortex showing that input signals coding for multiple stimulus orientations are present in each neuron, regardless of its output tuning (24), and *in vitro* work showing that particular dendrites can propagate inputs to the soma more effectively than others (25) via dendritic spiking (15, 16, 25, 26).

Our results strongly support such nonlinear dendritic amplification mechanisms, regardless of whether synaptic inputs are uniformly distributed. In particular, *in vitro* work has shown that depolarizing the somatic (27, 28) or dendritic (29) V_m can gate inputs in a thresholdlike manner. The lack of a hill or increased fluctuations at resting V_m makes unlikely a purely somatic locus of amplification. Rather, somatic depolarization may (i) shift the dendritic V_m closer to the activation range of voltage-gated conductances or deactivate hyperpolarization-activated currents (increasing R_N locally), and thus amplify spatially tuned responses and trigger dendritic spikes, or (ii) simply push the peak response in the dendrite above the dendritic spike threshold. Or instead, depolarization may act to let already existing dendritic spikes propagate to the soma (29).

These findings have important implications for place cells, spatial memory, and hippocampal-dependent memory in general. In novel environments, inhibitory neuron activity drops (30), which could depolarize the somatic baseline V_m of a silent cell and produce place fields (30, 31) without requiring synaptic plasticity. Neuromodulation could also cause depolarization or lower the threshold baseline $V_{m, \text{gate}}$ described here. Furthermore, already existing place cells could be those neurons whose $V_{m, \text{gate}}$ is below their baseline V_m . That is, (baseline $V_m - V_{m, \text{gate}}$) could reflect intrinsic excitability, with depolarization compensating for lower excitability in silent cells. Alternatively, place cell inputs could differ or be arranged differently on the dendritic tree, although this would not easily explain preexploration differences in excitability (14). Finally, the explicit demonstration that most CA1 pyramidal neurons, even originally silent ones, can be place cells in a given maze suggests that the role

of CA1 is not only to create spatial tuning, but to choose which subset of neurons should be active within a specific environment. The exclusively input-based models of place cells (7–11) could work as described but with an independent, excitability-based “AND” gate added. The distribution of excitability levels across the population would then determine which cells would represent a new item or environment in memory (14, 32). Moreover, the gating machinery would be a potential locus of plasticity for long-term storage (25–29).

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Supplementary Materials

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Materials and Methods

Figs. S1 to S10

References

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Neural Representations of Location Composed of Spatially Periodic Bands

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The mammalian hippocampal formation provides neuronal representations of environmental location, but the underlying mechanisms are poorly understood. Here, we report a class of cells whose spatially periodic firing patterns are composed of plane waves (or bands) drawn from a discrete set of orientations and wavelengths. The majority of cells recorded in parasubicular and medial entorhinal cortices of freely moving rats belonged to this class and included grid cells, an important subset that corresponds to three bands at 60° orientations and has the most stable firing pattern. Occasional changes between hexagonal and nonhexagonal patterns imply a common underlying mechanism. Our results indicate a Fourier-like spatial analysis underlying neuronal representations of location, and suggest that path integration is performed by integrating displacement along a restricted set of directions.

Grid cells represent the animal's location by firing in a hexagonally symmetric array of locations covering the entire environment (1). These cells are found in the medial entorhinal cortex (mEC) (1, 2) and in pre- and parasubiculum

(PaS) (2). This spatially periodic firing may provide the spatial metric for the hippocampal cognitive map (3–6). The hexagonal symmetry raises important questions: Is this an entirely unique pattern or one end of a continuum? Is this pattern required for spatial representation, or does it reflect properties such as stability (7) or coding efficiency (8)? We recorded 351 cells from superficial layers (II and III) of the medial part of dorsocaudal mEC (five implants) and adjacent PaS (two implants) in seven adult male rats (fig. S1) while they foraged for food in a square enclosure (1.69 m²). Many of these showed the regular hexagonal

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pattern characteristic of grid cells [26% passed the standard “gridness” measure (*I*)] (Fig. 1A), but a surprisingly large portion (44%) had stable multi-peaked patterns lacking the signature hexagonal symmetry (Fig. 1, B and C).

We used two-dimensional (2D) Fourier spectral analysis to identify cells whose spatial firing patterns showed significant spatial periodicity (9), being predominantly composed of a small number of Fourier components (that is, periodic spatial bands with different wavelengths and orientations that sum to produce the firing-rate map) (Fig. 1F). A cell was categorized as spatially periodic if its strongest Fourier component exceeded

95% of those in spatially shuffled data (fig. S2). Overall, 70% of cells were spatially periodic (Fig. 1E and fig. S3, all cells). Of these, 37% were grid cells, which usually have three main Fourier components with similar wavelengths, oriented at multiples of 60° from each other (Fig. 1, F and G, and figs. S4 to S8). The remaining spatially periodic cells had one to four main Fourier components with a greater range of relative orientations and wavelengths (Fig. 1G, figs. S6 and S7, and see fig. S8 for comparability of physiological properties). Spatial periodicity might reflect local rather than global spatial structure in the multi-peaked firing fields. Accordingly, we used a sec-

ond shuffling technique based on shuffling local peak-centered segments of the data. The results show that 144 of 154 (94%) spatially periodic non-grid cells and 89 of 91 (98%) grid cells had significantly more spatially periodic firing patterns than the $P = 0.05$ level in these shuffled data [that is, significantly more global periodicity than predicted by their local spatial structure ($P < 0.01$, binomial probability distribution) (fig. S9) (9)]. Several cells with one or two main Fourier components (for example, see Fig. 1C and fig. S10) were reminiscent of the band cells postulated as inputs to grid cells in some computational models [(7, 10), see also (11–13)].

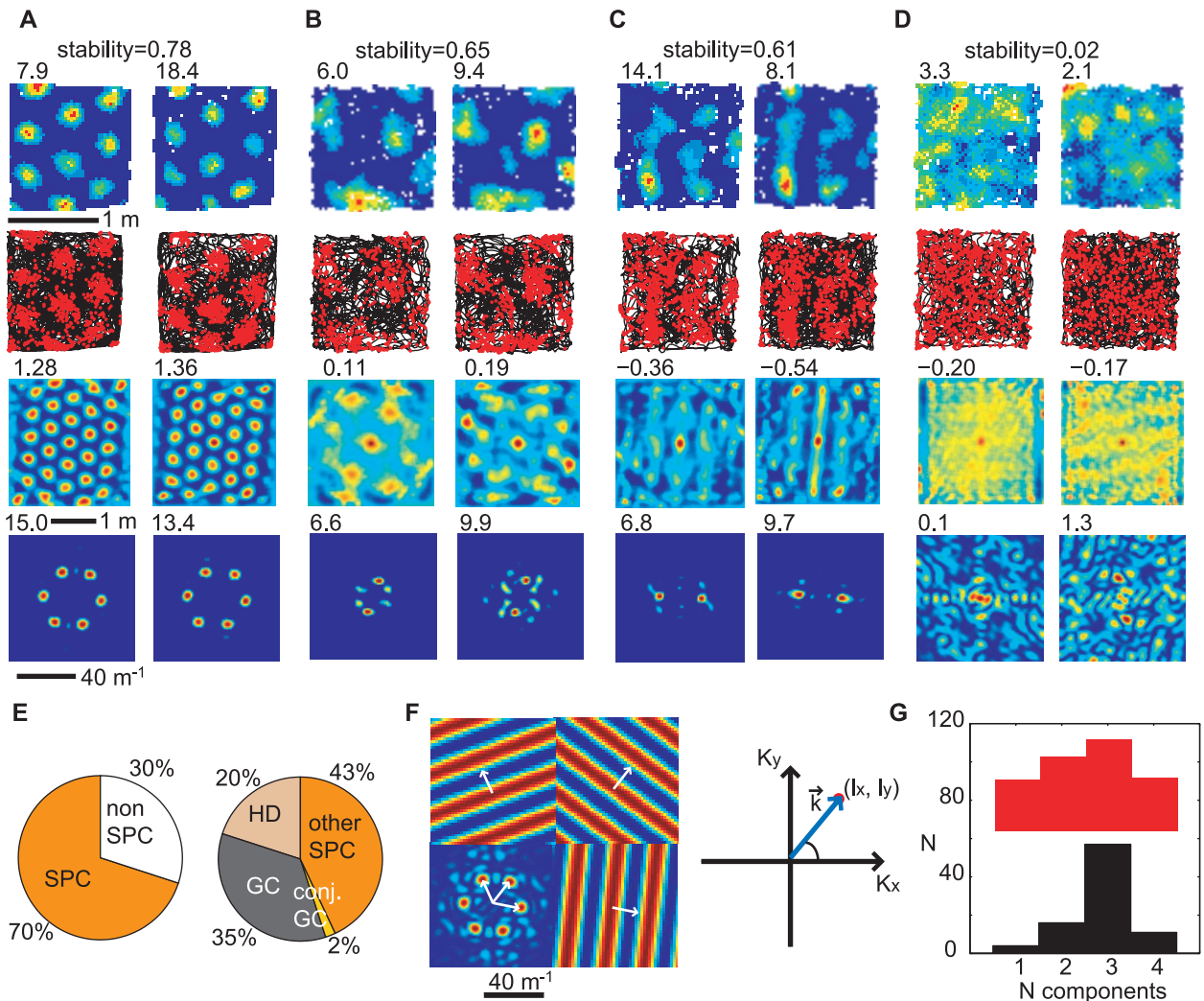


Fig. 1. Spatial periodicity of neuronal firing patterns. (A to D) Firing-rate maps (top row), trajectory (black) with spike positions (red) (second row), spatial autocorrelograms (third row), and 2D Fourier spectrograms (bottom row) for two successive trials of the same cell in a 1.3- by 1.3-m² enclosure. (A to C) Spatially periodic cells. The cell in (A) qualifies as a grid cell, the cell in (B) fires in an irregular grid, and that in (C) has a more bandlike firing pattern. (D) A non spatially periodic cell. Peak firing rate, gridness, and maximum Fourier power are shown (top left of the corresponding plots). Rate-map stability between trials is indicated above. (E) Distribution of cell types in dorsal mEC and adjacent PaS. (Left) All cells, divided into spatially periodic (SPC) and non-spatially periodic (nonSPC) cells. (Right) Spatially

periodic cells only, divided into grid cells (GC), conjunctive grid cells (conj. GC), SPCs with a head-direction correlate (HD), and other SPCs. (F) Two-dimensional Fourier analysis. (Left) The centered 2D Fourier spectrogram of the rate map shown at left in (A), with the main Fourier components shown at the sides with corresponding wave vectors (white arrows). (Right) The spectrogram shows the power corresponding to plane waves (wave vector $\mathbf{k}$) at (l_x, l_y) from the center. The periodic bands in the plane wave are oriented perpendicular to the wave vector $\mathbf{k}$, and their wavelength is inversely proportional to its length. (G) Distribution of the number of main Fourier components across spatially periodic cells (nongrid cells shown in red, grid cells in black).

Could the spatially periodic nongrid cells provide a consistent metric for an environment comparable to the grid cells? First, we looked at the stability of their firing patterns in familiar environments, between successive trials on the same day and between trials on different days. Spatially periodic nongrid cell firing patterns were significantly more stable than chance, both within and between days. However, grid-cell firing patterns were even more stable than nongrid spatially periodic cells on both comparisons (Fig. 2A). The greater stability of grid-cell firing patterns corresponded to greater stability in the orientations of their Fourier components (Fig. 2B), suggesting a causal relationship between the two.

Some spatially periodic cells (11%) changed their firing patterns from grids to nongrids or vice versa between trials in the same environment (figs. S11 and S12 and table S1). Figure 2C

shows an example of a cell whose firing pattern changed from gridlike to bandlike (a simultaneously recorded grid cell remained unchanged, see fig. S12 for details). Such transitions did not reflect unidirectional drift of the grid pattern (sliding time-window spatial autocorrelation analysis) (fig. S13). These transitions suggest a continuous population of spatially periodic cells, with grids and nongrids reflecting different combinations of a small set of elemental periodic bands.

A larger proportion of spatially periodic cells (32%) changed between grids and nongrids across different environments (Fig. 2D and table S1), although the majority did not change category (Fig. 2, E and F). These transitions corresponded to changing configurations of underlying periodic bands. They did not reflect simpler transformations previously reported for grid cells af-

ter environmental manipulations [rotations and translations (14), rescaling (15), or expansions (16)].

Are the spatially periodic cells in one animal composed from the same set of bands? All of these cells tended to have Fourier components clustered around a small number of orientations and wavelengths (figs. S5 to S7) (15). As expected, grid-cell components were aligned (15, 17) and oriented at 60° to each other (Fig. 3, A to G; Rayleigh vector $R_0 = 0.58 \pm 0.14$ for clustering modulo 60° , $P < 0.01$), corroborated by a clustering of the angular separation of neighboring components around 60° (Fig. 3H; Rayleigh vector $R_0 = 1.1$, $P < 0.001$). The orientations of components of spatially periodic nongrid cells tended to align with those of grid cells (Fig. 3, A to G, and fig. S5; mean correlation coefficient of 0.54 ± 0.08 , $P < 0.01$ from shuffled data) but included a wider range of relative orientations (Fig. 3I).

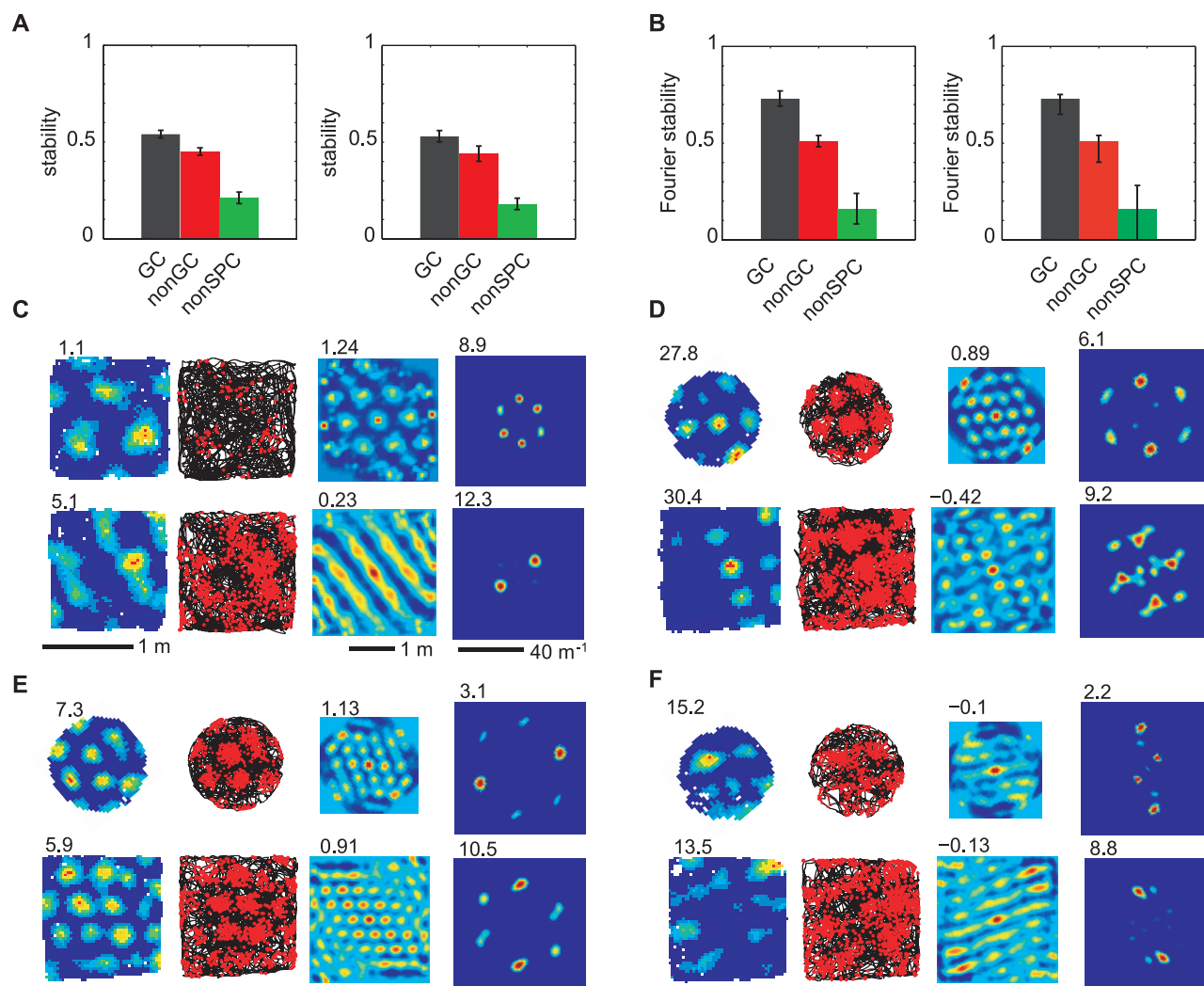


Fig. 2. Stability of spatially periodic cell firing. **(A)** Grid cells were more stable than spatially periodic nongrid cells (nonGC), and both were superior to nonspatially periodic cells, both between sessions on the same day in the same environment [(left) GC: correlation coefficient $r = 0.54 \pm 0.02$ (mean $\pm$ SEM), nonGC: $r = 0.45 \pm 0.02$, nonSPC: $r = 0.21 \pm 0.03$, $P_{144}(\text{GC} > \text{nonGC}) = 0.015$, $P_{78}(\text{GC} > \text{nonSPC}) = 0.65\text{E}-012$, $P_{125}(\text{nonGC} > \text{nonSPC}) = 1.69\text{E}-010$, paired t tests, one-tailed] and across days [(right) GC: $r = 0.53 \pm 0.03$, nonGC: $r = 0.44 \pm 0.04$, nonSPC: $r = 0.18 \pm 0.03$, $P_{51}(\text{GC} > \text{nonGC}) = 0.049$, $P_{24}(\text{GC} >$

nonSPC) = $1.39\text{E}-005$, $P_{41}(\text{nonGC} > \text{nonSPC}) = 3.14\text{E}-004$; paired t tests, one-tailed]. **(B)** Fourier polar-component stability mirrored rate-map stability between sessions [(left), GC > nonGC: $r = 0.73 \pm 0.04 > r = 0.51 \pm 0.03$, $P_{144} = 3.24\text{E}-005$, paired t tests, one-tailed] and between days [(right) 0.70 ± 0.06 versus 0.47 ± 0.07 , $P_{24} = 0.015$, paired t tests, one-tailed]. Occasionally, cell firing altered between grid and nongrid patterns between trials **(C)** in the same environment or **(D)** in different environments. More often, the structure of grids **(E)** and spatially periodic nongrids **(F)** was maintained across environments.

Fig. 3. Orientations of main Fourier components within each animal. **(A to G)** Histograms of the orientations of main Fourier components of the spatially periodic cells recorded in each animal (in a 1.3- by 1.3-m² enclosure; nongrid cells shown in red, grid cells in black). **(A and B)** cells in PaS; **(C to G)** cells in mEC [**(A)** r1682, **(B)** r1738, **(C)** r1728, **(D)** r1709, **(E)** r1737, **(F)** r1739, **(G)** r1710]. Distributions are shown for the relative orientations of Fourier components of all grid cells **(H)** and all spatially periodic nongrid cells **(I)**.

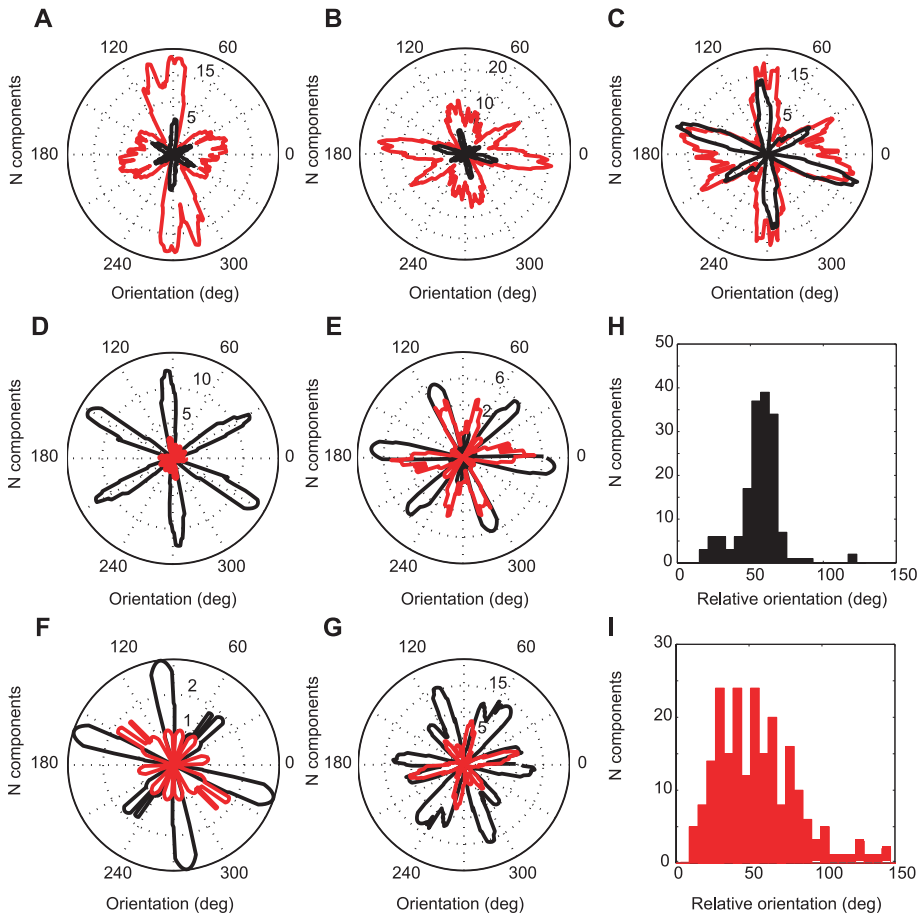
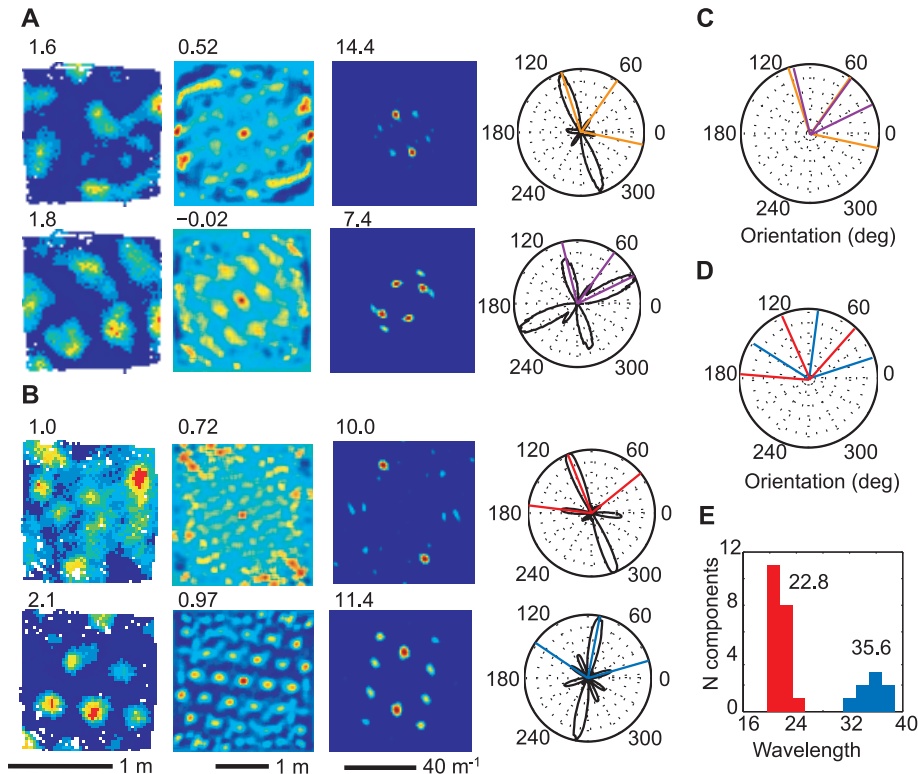


Fig. 4. Spatially periodic cells with different Fourier components coexist within the same animal. **(A)** Simultaneously recorded grid cell (top) and spatially periodic nongrid cell (bottom) with one Fourier component misaligned between the two (r1738). **(B)** Simultaneously recorded grid cells of different scale and orientation (r1737). (Left to right) Firing rate map, spatial autocorrelogram, 2D Fourier spectrogram with peak firing rate, gridness score, and maximum Fourier power indicated on the top-left of the corresponding plots are shown. **(C)** Orientations of the main Fourier components in **(A)** are superimposed here [colors as in **(A)**]. **(D)** Mean orientations of the main Fourier components (fig. S15) in **(B)** [colors as in **(B)**]. **(E)** Wavelength distribution of Fourier components of simultaneously recorded grid cells with the same orientations as the cell in **(B)**, top; red and the cell in **(B)**, bottom; blue. The mean wavelength for each module is indicated above. Their ratio is ~ 1.57 .



If grid cells and spatially periodic nongrid cells are composed of subsets from a larger set of bandlike components, there should be examples of simultaneously recorded cells with different components. Figure 4, A and C, shows a grid and nongrid spatially periodic cell differing by $\sim 30^\circ$ in the orientation of one of their components (see fig. S14 for more examples). Figure 4, B and D, shows two simultaneously recorded grid cells whose three component orientations all differ by $\sim 30^\circ$ and also differ in wavelengths (see fig. S15 for details).

Finally, we asked whether the proportions of grid and spatially periodic nongrid cells differed between the two anatomical regions investigated. Although the overall percentage of spatially periodic cells was lower in mEC (156/239, 65% in mEC; 89/112, 79% in PaS), the proportion of grid cells in mEC (75/156 or 48%) was much higher than in PaS (16/89 or 18%), despite the close anatomical proximity of these regions (fig. S1). This observation suggests that both regions, with very distinct afferent and efferent connectivity (2, 18, 19), can generate spatially periodic cells, albeit with major differences in the prevalence of pure hexagonal symmetry. In both regions, cell firing shows strong theta modulation: 67% of all spatially periodic cells were theta-modulated in PaS (mean frequency 9.2 ± 0.1 Hz; 65% of all cells were theta-modulated) and 56% in mEC (with similar mean frequency; 47% of all cells were theta-modulated), pointing to a common characteristic of spatially periodic cells regardless of their anatomical location (fig. S16).

The abrupt increase in the proportion of grid cells in mEC suggests that mEC has microcircuitry or anatomical inputs organized to prefer components at 60° angles, which, in turn, provides the most stable inputs to the hippocampus. In contrast, PaS, with a higher proportion of spatially periodic cells but more varied and less stable firing patterns, heavily projects to the superficial layers of mEC (18–20) and may represent an intermediate stage in constructing stable grids from periodic bands.

The heterogeneous spatially periodic firing patterns reported here could reflect a common process of self-organization acting on band cells upstream of the recorded cells (7, 10). The most stable outcome of such a process would be hexagonal grids (10), though mixtures of orientations organized at multiples of around 30° and 60° can also be stable (7) and may support multiscale representation (4, 21). What mechanism might underlie the bands? Theta-modulated firing reminds us that theta rhythmicity controls the scale of spatial representations (22–24) and that interference between theta oscillations could integrate self-motion to produce bandlike firing patterns that drive grid-cell firing (10–12). These band cells might correspond to phase-modulated firing from theta cells in the septohippocampal circuit (13) or may be generated by 1D ring attractors (7, 25). Alternatively, in the attractor model (4, 6, 26), recurrent interactions between grid cells could produce a stable gridlike, as well as periodic bandlike Turing pattern

(6, 26, 27) of firing across a sheet of cells, which translates into spatial firing as the animal moves.

In summary, the firing patterns of the majority of cells recorded in PaS and mEC showed a stable spatial periodicity described by superposition of a small number (up to four) of elemental periodic bandlike components drawn from a discrete set of orientations and wavelengths. Grid cells correspond to hexagonal configurations, which have the greatest spatial stability. Our results suggest that path integration is performed by integrating self-motion along a restricted set of directions, mediated by planar periodic representations (bands) of multiple scales along each direction, and that self-organization of these bandlike inputs may underlie the firing of all spatially periodic cells in the parahippocampal region.

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Supplementary Materials

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Materials and Methods

Figs. S1 to S16

Table S1

References

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A TOG: $\alpha\beta$ -tubulin Complex Structure Reveals Conformation-Based Mechanisms for a Microtubule Polymerase

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Stu2p/XMAP215/Dis1 family proteins are evolutionarily conserved regulatory factors that use $\alpha\beta$ -tubulin-interacting tumor overexpressed gene (TOG) domains to catalyze fast microtubule growth. Catalysis requires that these polymerases discriminate between unpolymerized and polymerized forms of $\alpha\beta$ -tubulin, but the mechanism by which they do so has remained unclear. Here, we report the structure of the TOG1 domain from Stu2p bound to yeast $\alpha\beta$ -tubulin. TOG1 binds $\alpha\beta$ -tubulin in a way that excludes equivalent binding of a second TOG domain. Furthermore, TOG1 preferentially binds a curved conformation of $\alpha\beta$ -tubulin that cannot be incorporated into microtubules, contacting α - and β -tubulin surfaces that do not participate in microtubule assembly. Conformation-selective interactions with $\alpha\beta$ -tubulin explain how TOG-containing polymerases discriminate between unpolymerized and polymerized forms of $\alpha\beta$ -tubulin and how they selectively recognize the growing end of the microtubule.

Microtubules are highly regulated, dynamic polymers of $\alpha\beta$ -tubulin that have essential roles in intracellular organization and chromosome segregation. Microtubules grow faster in vivo than they do in vitro [reviewed in (1)]. Proteins in the Stu2p/XMAP215/Dis1 family (2–4) are the major cellular factors that promote fast microtubule elongation. These proteins contain multiple tumor overexpressed gene (TOG)

domains that function as $\alpha\beta$ -tubulin binding modules (5) and are required for the elongation-promoting activity of this family (5, 6). The

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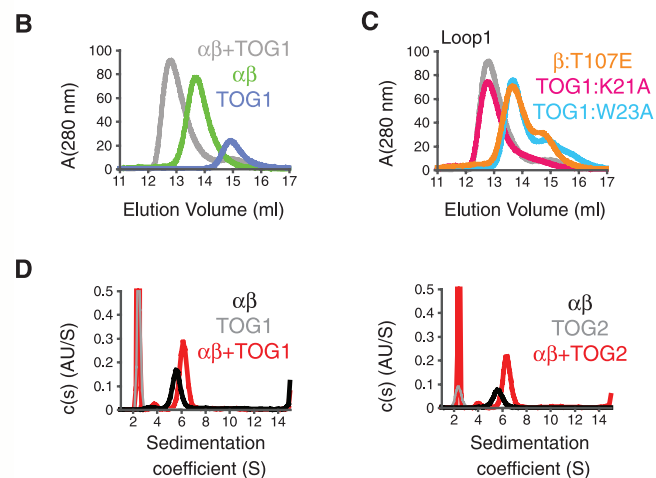
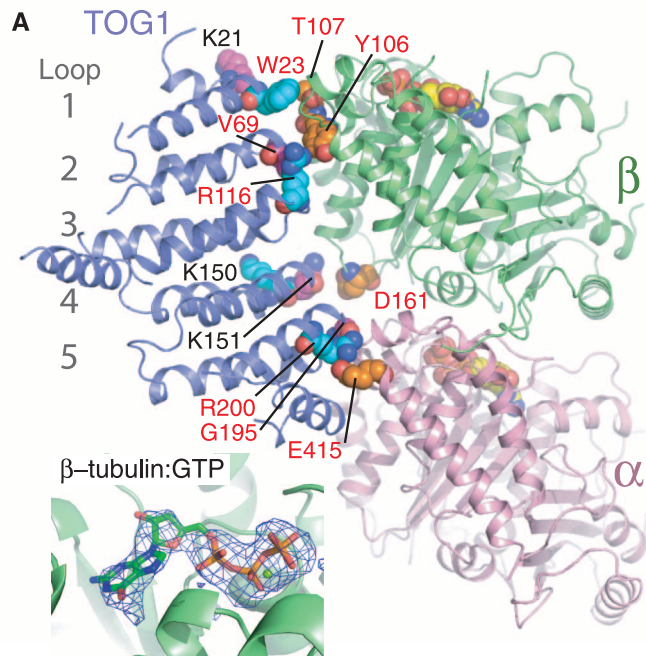


Fig. 1. Structure of a TOG1:αβ-tubulin complex, revealing noteworthy contacts with α- and β-tubulin. **(A)** Cartoon representation of the complex (pink, α-tubulin; green, β-tubulin; blue, TOG1). Contacts probed by mutagenesis are represented as spheres [and are colored to match (C) and fig. S3], as are GTPs. Y, Tyr; G, Gly. (Inset) mF_o-DF_c omit electron density map contoured at 3.5σ and computed from a model without nucleotides: β-tubulin is bound to GTP. **(B)** Size-exclusion chromatography assay for TOG1:αβ-tubulin interactions. A, absorbance. **(C)** TOG1:αβ-tubulin binding assay using interface mutants on loop 1 (fig. S3). **(D)** TOG1 (left) and TOG2 (right) (gray) each form a 1:1 complex (red) with αβ-tubulin (black), as detected by analytical ultracentrifugation (table S4). Curves are shown from s = 1 S to eliminate a slowly sedimenting contaminant in the TOG2 run. c(s), signal population as a function of s; AU, absorbance units.

molecular mechanisms underlying the activity of these microtubule polymerases are poorly understood. Recent studies of XMAP215 suggest that it functions as a catalyst, using at least two TOG domains (6, 7).

The ability to function as a catalyst for microtubule elongation suggests that XMAP215/Stu2p family proteins use their tubulin-binding TOG domains to distinguish between at least three different forms of αβ-tubulin: unpolymerized, in the body of the microtubule, and at the growing end of the microtubule. However, the mechanism by which these proteins discriminate has remained unclear. To establish the structural basis of TOG:tubulin recognition, we determined the crystal structure of the TOG1 domain from Stu2p bound to αβ-tubulin (Fig. 1A). We used a polymerization-blocked mutant of yeast αβ-tubulin to obtain crystals (8, 9). The structure was determined by molecular replacement from crystals that diffracted anisotropically to 2.88 Å (minimum Bragg spacing of 3.44 Å in the weakly diffracting direction, overall completeness of 74.6%) (tables S1 and S2). The structure contains guanosine triphosphate (GTP) at the exchangeable nucleotide-binding site of αβ-tubulin (Fig. 1A, inset).

TOG1 forms a flat, layered structure similar to that observed for other TOGs (Fig. 1A and fig. S1) (10, 11). Almost the entire narrow, evolutionarily conserved face of TOG1 (10, 11) interacts with αβ-tubulin (Fig. 1A and fig. S1), burying ~1600 Å² of surface area, 58% of which is attributable to the partial interface with β-tubulin. The asymmetric mode of TOG1 binding apparently excludes analogous binding of TOG2 to the same heterodimer. This finding is unexpected be-

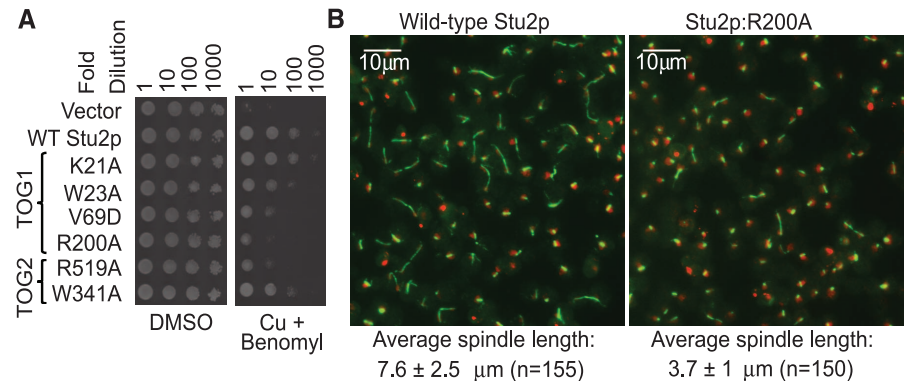


Fig. 2. Disruptive point mutations on the tubulin-binding interfaces of TOG1 or TOG2 affect Stu2p function in vivo. **(A)** Yeast carrying plasmid-based rescue constructs of Stu2p were plated at serial dilutions on media containing dimethyl sulfoxide (DMSO) (control) or 500 μM CuSO₄ (to deplete endogenous Stu2p) plus 20 μg/ml benomyl (to cause microtubule stress). TOG1 or TOG2 impaired for αβ-tubulin interactions only partially compensate for the depletion of endogenous Stu2p. **(B)** Fluorescence images at 1 hour after release from hydroxyurea arrest (green, αβ-tubulin; red, DNA) of yeast depleted of endogenous Stu2p and rescued with wild-type (left) or R200A (right) Stu2p. Spindle elongation is compromised when TOG1:αβ-tubulin interactions are impaired (R200A). n, number of spindles measured.

cause it was assumed that multiple TOGs could simultaneously engage a single αβ-tubulin heterodimer (5, 7, 10). We used site-directed mutagenesis and a gel-filtration binding assay to probe the importance of TOG1:αβ-tubulin contacts (Fig. 1, B and C, and figs. S2 and S3) (10, 11). TOG1:αβ-tubulin interactions were affected by mutations on α- or β-tubulin-contacting loops of TOG1 or on contacted surfaces of α- or β-tubulin [e.g., W23A (Trp²³→Ala²³) or R200A (R, Arg) on TOG1, T107E (T, Thr; E, Glu) on β-tubulin, E415A on α-tubulin] (Fig. 1, A and C). The importance of

TOG1:W23 confirms findings from earlier studies (10, 11). The salt bridge between TOG1:R200 and α-tubulin:E415 (fig. S4) that is required for robust TOG1:αβ-tubulin interactions (Fig. 1C) rationalizes the strong evolutionary conservation of R200 in TOG domains (10). Analytical ultracentrifugation revealed that TOG1 and TOG2 each bind αβ-tubulin efficiently at low micromolar concentrations (Fig. 1D). Thus, TOG1:αβ-tubulin interactions detected in solution require simultaneous engagement with both α- and β-tubulin as observed in the crystal, and TOG2 can interact with unpolymerized αβ-tubulin.

Fig. 3. $\alpha\beta$ -tubulin–GTP adopts a curved conformation. (A) Superposition of yeast α - (pink) and β -tubulin (green) shows similar positioning of the H6–H7 segment (represented with darker colors). (B) Superposition of yeast α - and β -tubulin onto curved (α , orange; β , yellow) and straight (α , maroon; β , dark blue) structures shows the H6–H7 segments of yeast tubulins arranged as in earlier curved structures. (C) Pairwise $C\alpha$ root mean square coordinate deviations (rmsd) between yeast α - and β -tubulin and earlier structures, computed for the subdomains indicated (table S3). (D) The quaternary structure of yeast $\alpha\beta$ -tubulin (pink and green) resembles that of the curved form (gray, left) and differs from the straight form (gray, right).

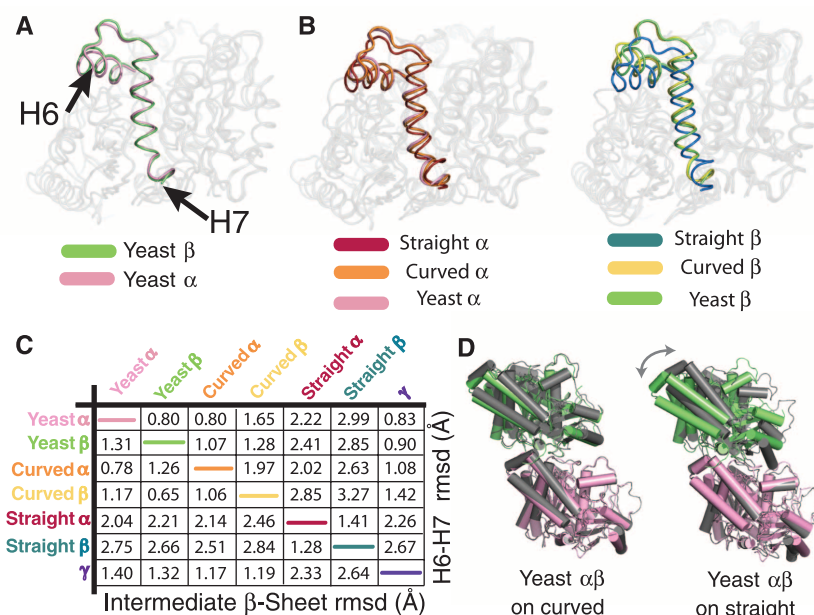
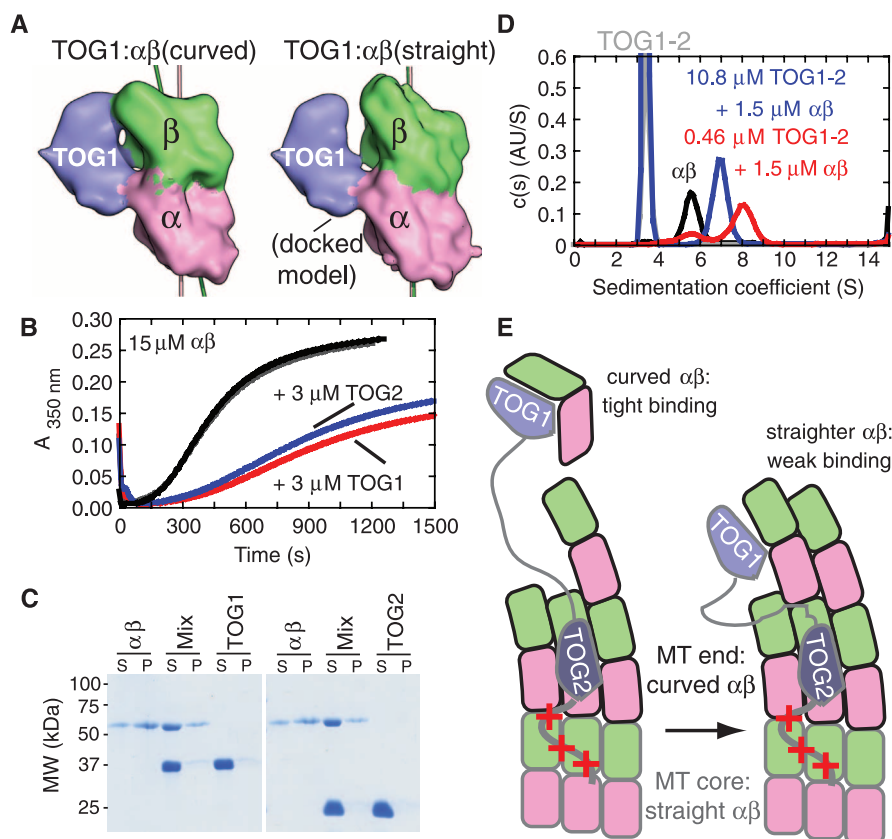


Fig. 4. TOG1: $\alpha\beta$ -tubulin interactions are conformation-selective. (A) The structure of the TOG1: $\alpha\beta$ -tubulin complex (left) and a docked model with straight $\alpha\beta$ -tubulin (right) illustrates how TOG1-contacting epitopes on α - and β -tubulin move relative to each other in the two conformations. (B) Microtubule-assembly reactions (15 μM animal $\alpha\beta$ -tubulin) containing 3 μM TOG1 (red) or TOG2 (blue) are inhibited relative to control reactions (black and gray) that received only buffer. (C) Microtubule cosedimentation showing that TOG1 and TOG2 do not appreciably bind microtubules, even though the TOG-interacting epitopes are accessible on the outside of the microtubule (fig. S7). MW, molecular weight; S, supernatant; P, pellet. (D) Size distributions showing that substoichiometric concentrations of TOG1–TOG2 mixed with $\alpha\beta$ -tubulin (red) behave as a complex that sediments faster than $\alpha\beta$ -tubulin alone (black) and the TOG1–TOG2:($\alpha\beta$)₁ complex that results when TOGs are in molar excess over $\alpha\beta$ -tubulin (blue). (E) Minimal cartoon model illustrating how conformation-selective TOG: $\alpha\beta$ -tubulin interactions contribute to function. “+++” denotes a basic region that provides microtubule affinity. TOG1 can efficiently capture unpolymerized $\alpha\beta$ -tubulin in its naturally curved state (left), but binds less tightly to straight/straighter conformations of $\alpha\beta$ -tubulin in the microtubule (right). TOG2 may recognize an end-specific conformation of $\alpha\beta$ -tubulin.



We used conditional depletion of Stu2p (12) and a rescue assay to investigate how mutations on the tubulin-interacting surfaces of TOG1 or TOG2 affect Stu2p function in vivo. Wild-type Stu2p and Stu2p with a TOG1 mutation (K21A; K, Lys) that did not interfere with $\alpha\beta$ -tubulin binding completely rescued the growth defect arising from the depletion of endogenous Stu2p. In contrast, Stu2p with mutations in TOG1 [W23A, V69D (V, Val; D, Asp), or R200A] that affect $\alpha\beta$ -

tubulin binding was compromised for rescue (Fig. 2A). Mutations on the presumptive $\alpha\beta$ -tubulin-interacting surface of TOG2 (W341A and R519A) affected rescue similarly to their TOG1 equivalents (W23A and R200A) (Fig. 2A). The R200A mutant of Stu2p displayed a defect in mitotic spindle elongation (Fig. 2B), similar to the complete removal of the TOG1 domain (5).

The conformations of α - and β -tubulin in the TOG1 complex are remarkably similar to each

other (Fig. 3, A and B, and fig. S5), the curved α - and β -tubulin monomer conformations previously described (13), and γ -tubulin (Fig. 3B and figs. S5 and S6) (14). A 13° rotation is required to superimpose the α - and β -tubulin chains in the TOG1 complex. This quaternary arrangement also closely resembles that of a curved heterodimer (12° rotation) (13), which is characteristically distinct from the straight heterodimer (~1° rotation) (Fig. 3, C and D) (15). Instead, this curved conformation

could represent a conserved ground state of $\alpha\beta$ -tubulin. Together, these observations add further support to a model in which the role of GTP is to promote assembly by tuning the strength of polymerization contacts (16) and/or by decreasing the free-energy difference between straight and curved conformations (17).

The regions of curved $\alpha\beta$ -tubulin that engage TOG1 move relative to each other in the transition to the straight conformation (Fig. 4A). Thus, TOG1: $\alpha\beta$ -tubulin interactions might be sensitive to $\alpha\beta$ -tubulin quaternary structure, with a preference for curved $\alpha\beta$ -tubulin. If TOG1 binds preferentially to curved $\alpha\beta$ -tubulin, it should inhibit in vitro microtubule formation by stabilizing a microtubule-incompatible conformation of $\alpha\beta$ -tubulin. We used microtubule-assembly reactions to test this counterintuitive prediction, and we observed strong inhibition when TOG1 was present (Fig. 4B and fig. S8), consistent with earlier observations (11). We did not observe inhibition for TOG1 mutants (e.g., W23A or R200A) (fig. S9) that affect $\alpha\beta$ -tubulin binding. TOG1 does not bind appreciably to straight $\alpha\beta$ -tubulin in preformed microtubules (Fig. 4C), despite the apparent accessibility of the TOG1-interacting epitopes on the outside of the microtubule (fig. S7). Thus, TOG1 binds preferentially to curved $\alpha\beta$ -tubulin. We obtained similar results for TOG2 (Fig. 4, B and C), indicating that it also binds preferentially to an $\alpha\beta$ -tubulin conformation that cannot exist in the body of the microtubule.

TOG2 binds to GTP- or guanosine diphosphate-bound $\alpha\beta$ -tubulin with approximately equal affinity (200 to 300 nM) (fig. S10), supporting a model in which the curvature of unpolymerized $\alpha\beta$ -tubulin does not change appreciably as a function of the bound nucleotide. For the “hand-off” to the microtubule to be efficient, the affinity of $\alpha\beta$ -tubulin: microtubule interactions must at least be comparable to that of TOG: $\alpha\beta$ -tubulin interactions. We used analytical ultracentrifugation to demonstrate that TOG1-TOG2 and $\alpha\beta$ -tubulin interact in a manner that is most consistent with a fast interchange between 1:1 and 1:2 TOG1-TOG2: $\alpha\beta$ -tubulin complexes (Fig. 4D, red trace). The observation of a TOG1-TOG2:($\alpha\beta$ -tubulin)₂ complex is surprising, because earlier studies (5, 7) suggested that multiple TOG domains could simultaneously engage the same $\alpha\beta$ -tubulin. Some of these earlier studies were conducted using a gel-filtration binding assay similar to the one we used, so it is possible that complexes with multiple $\alpha\beta$ -tubulins were overlooked [we initially overlooked TOG2: $\alpha\beta$ -tubulin interactions for the same reason (fig. S2)]. Our data also show that the complex formed depends on the relative stoichiometry of TOG domains to $\alpha\beta$ -tubulin.

We hypothesize that the structure we determined provides a model for substrate recognition in which TOG1 [which is dispensable for plus-end binding (5)] of microtubule-bound Stu2p would capture unpolymerized subunits and/or stabilize a collision complex through its relatively strong interactions with naturally curved $\alpha\beta$ -tubulin (Fig. 4E). Selective microtubule-end association is pre-

sumably the combined effect of a basic region in Stu2p providing microtubule lattice affinity (5) and TOG2 preferentially recognizing an end-specific conformation of $\alpha\beta$ -tubulin. We speculate based on the polarity of TOG: $\alpha\beta$ -tubulin engagement that the ordering of TOGs and the basic region dictates plus-end specificity. For TOG2 and the C-terminal basic domain to jointly mediate plus-end recognition, they must be able to engage the microtubule in a way that allows TOG2 to bind nonstraight $\alpha\beta$ -tubulins at the very end and the basic region to simultaneously contact surfaces deeper in the polymer. This can only occur at the plus end. The conformational straightening in $\alpha\beta$ -tubulin that accompanies lattice incorporation will result in lower-affinity TOG1 interactions (Fig. 4E). In this hand-off mechanism, polymer incorporation and release of TOG1 for a subsequent round of capture would be intrinsically coupled by virtue of the conformational preferences of TOG1. Hand-off will become efficient only when TOG1 is tethered to free $\alpha\beta$ -tubulin binding sites at the end of the microtubule; this explains the requirement for at least two TOGs (6), as well as why isolated TOG1 or TOG2 inhibits microtubule assembly despite functioning to promote assembly when part of Stu2p.

Collectively, our observations indicate that Stu2p/XMAP215 family proteins use conformation-selective TOG: $\alpha\beta$ -tubulin interactions to discriminate between unpolymerized and polymerized forms of $\alpha\beta$ -tubulin. By extension, this result suggests that assembly dependent conformational change in $\alpha\beta$ -tubulin plays an important role in dictating microtubule polymerization dynamics.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/337/6096/857/DC1
Materials and Methods
Figs. S1 to S10
Tables S1 to S4
References (18–27)

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A Mechanism of Extreme Growth and Reliable Signaling in Sexually Selected Ornaments and Weapons

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Many male animals wield ornaments or weapons of exaggerated proportions. We propose that increased cellular sensitivity to signaling through the insulin/insulin-like growth factor (IGF) pathway may be responsible for the extreme growth of these structures. We document how rhinoceros beetle horns, a sexually selected weapon, are more sensitive to nutrition and more responsive to perturbation of the insulin/IGF pathway than other body structures. We then illustrate how enhanced sensitivity to insulin/IGF signaling in a growing ornament or weapon would cause heightened condition sensitivity and increased variability in expression among individuals—critical properties of reliable signals of male quality. The possibility that reliable signaling arises as a by-product of the growth mechanism may explain why trait exaggeration has evolved so many different times in the context of sexual selection.

The most elaborate male ornaments and weapons of sexual selection grow to exaggerated proportions (Fig. 1), especially in the largest and best-conditioned individuals.

The size and conspicuousness of these traits make them likely candidates for intraspecific signals, used either by males to assess the size, condition, or status of rival males, or by females to assess the

relative genetic quality of potential mates (1, 2). Not only are exaggerated traits easy to observe, they are unusually reliable signals of individual male quality (2–4), as their growth tends to be more sensitive to the nutritional histories and physiological conditions of individuals than is the growth of other traits (5–7). Exaggerated structures also tend to be more variable in their expression than other morphological structures (8–10). Hypervariability in trait size can amplify otherwise subtle differences in the body size or condition of males, further enhancing the utility of these traits as signals. Combined, these structural characteristics—extreme size, heightened condition sensitivity, and

hypervariability among individuals—are the foundation for “handicap” and “good genes” models of sexual selection and a central tenet of modern theories of sexual selection and animal communication (2–4, 11–15). We offer a developmental explanation for this phenomenon. We suggest that the evolution of trait exaggeration involves increased sensitivity to insulin/IGF signaling within a growing structure, and we show why such a change in mechanism should also confer both heightened condition sensitivity and hypervariability to expression of the trait (Fig. 1B).

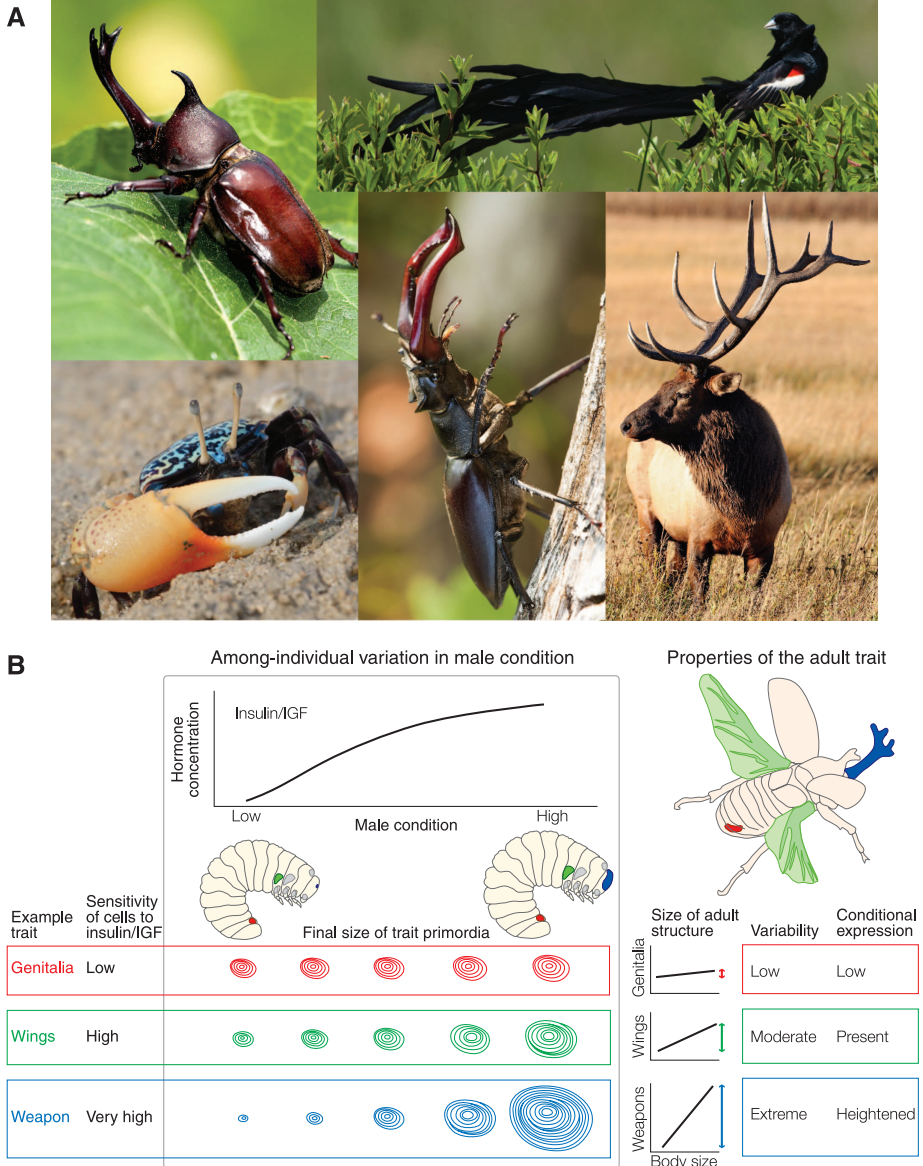
Insulin and IGFs are essential regulators of tissue growth and body size (16). Circulating concentrations of insulin and IGFs are sensitive to nutrition, as well as stress and infection, and the insulin/IGF pathway has emerged as the central mechanism integrating physiological condition with growth in multicellular animal taxa. Insulin and IGF levels within a growing animal reflect the nutritional state and physiological con-

dition of that individual, and circulating concentrations of these signals modulate tissue growth via the insulin receptor pathway in a graded, or dose-dependent, manner. Within an individual, growth will speed up or slow down in response to changes in nutritional or physiological state because of the action of this pathway. Across individuals, growth will differ between high-condition and low-condition individuals, resulting in population-level variation in body and trait sizes. Low-condition individuals have lower levels of these signals than higher-condition individuals, and as a result, they experience slower rates and lower overall amounts of tissue growth.

As long as the various organs and body parts (e.g., legs, eyes, wings) exhibit similar sensitivities to insulin/IGF signaling (17), their sizes will scale proportionally from individual to individual (18–21). But some traits deviate in their responsiveness to these signals, profoundly affecting the amount and nature of their growth. Genitalia are

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Fig. 1. (A) Exaggerated growth of weapons and ornaments of sexual selection. Clockwise from top left: Rhinoceros beetle horns (*Trypoxylus dichotomus*); long-tailed widowbird tail (*Euplectes progne*); elk antlers (*Cervus elaphus*); stag beetle mandibles (*Lucanus cervus*); fiddler crab chela (*Uca tetragonon*). **(B)** Proposed mechanism for the evolution of trait exaggeration through increased cellular sensitivity to insulin/IGF signaling (shown for the disc-like appendage primordia of insects). Individual nutritional state and physiological condition are reflected in circulating concentrations of insulin-like peptides and IGFs, which modulate the rate of growth of each of the trait primordia. Traits whose cells are sensitive (17) to these signals [e.g., wings (green)] exhibit greater nutrition-dependent phenotypic plasticity and among-individual variability than other traits whose cells are less sensitive to these signals [e.g., genitalia (red)]. An increase in the sensitivity of cells within a particular trait [e.g., horns (blue); see text] would lead to disproportionately rapid growth of that trait in the largest, best-condition individuals (i.e., exaggerated trait size) and smaller trait sizes in low-condition individuals.



insensitive to circulating insulin/IGF signals in *Drosophila* (20, 21). As a result, their growth is unresponsive to environmental conditions, such as nutrition, and genitalia size is largely invariant among individuals. In contrast, wings exhibit sensitivity to insulin/IGF signaling typical of the rest of the body; wing growth is sensitive to larval nutrition, and wing sizes scale isometrically with among-individual variation in body size (21).

We predicted that increased sensitivity to the insulin/IGF pathway might be a mechanism leading to the evolution of extreme growth in showy ornaments and weapons of sexual selection. In our model, individual males differ in their physiological state as a result of differences in their status, nutritional state, competitive ability, and/or health (parasite or pathogen loads), which translate into among-individual variation in circulating concentrations of insulin/IGF signals (Fig. 1B). During their respective periods of growth, the adult structures in these animals would be exposed to insulin/IGF signals, and the sensitivity of cells within each growing structure to these signals would determine both how and by how much each trait grew. Just as wings are more sensitive to insulin/IGF signaling than genitalia in *Drosophila* (20, 21), so we predicted that exaggerated ornaments or weapons of sexual selection would be even more sensitive to insulin/IGF signaling than wings or other non-sexually selected body parts (Fig. 1B).

Male rhinoceros beetles (*Trypoxylus dichotomus*) wield a forked horn on their heads. During growth, horns in this species are more sensitive to larval nutrition than other body parts (wings, genitalia), and among adult males, horn size is hypervariable, ranging from tiny bumps to exag-

gerated structures two-thirds the length of a male's body (22). We tested whether growing rhinoceros beetle horns were more sensitive to insulin/IGF signaling than wings or genitalia using RNA interference to perturb transcription of the insulin receptor (*InR*) gene. Developing larvae were injected with a 398-base pair fragment of double-stranded RNA (dsRNA) of *T. dichotomus InR* as they commenced their transition from larval feeding to gut purge (the onset of the prepupal period and the beginning of metamorphosis). At this time, all growth in overall body size had ceased, but adult structures (including genitalia, wings, and horns) were still growing. Thus, any effects of manipulation of insulin/IGF signaling would be visible as reductions to genitalia, wing, or horn size relative to overall body size. If the evolution of exaggerated horn size resulted in part from an increase in cellular sensitivity to insulin/IGF signaling, then horns should be more sensitive than wings to perturbation of the activity of this pathway. We also predicted that genitalia would be relatively insensitive to pathway perturbation [sensu (20, 21)].

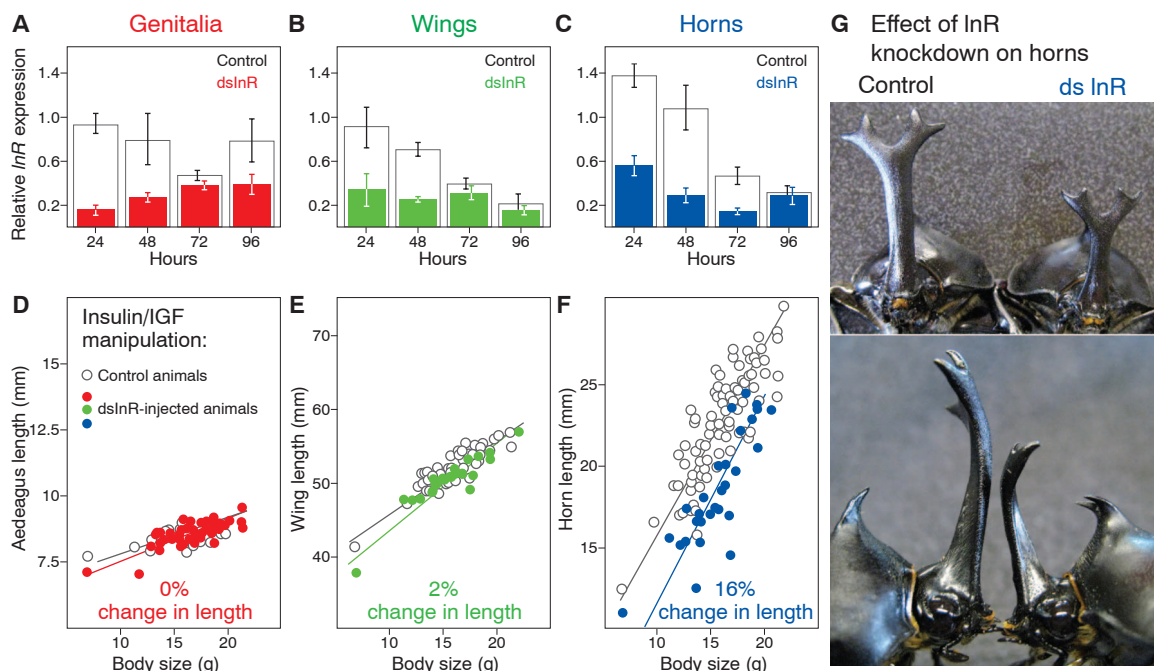
Injections significantly reduced *InR* transcript abundances for 48 hours near the end of the period of trait growth (i.e., before *InR* transcript abundance normally drops in these tissues; Fig. 2, A to C). After metamorphosis was completed, we compared morphologies of treated and control animals. Genitalia did not respond to experimental perturbation of *InR* pathway activity (Wald statistic = 0.1245, df = 1, $P = 0.724$; Fig. 2D). Wings, which exhibit nutrition-sensitive growth patterns typical of most metric traits (e.g., eyes, legs, elytra), showed a significant reduction in size of ~2% (Wald statistic = 8.976, df = 1, $P =$

0.003; Fig. 2E). In contrast, male horns, the structures most sensitive to nutrition, were reduced by ~16% relative to controls (Wald statistic = 68.37, 1 df, $P < 0.0001$; Fig. 2, F and G). Using response to *InR* knockdown as a metric, we found that male horns were eight times as sensitive to insulin/IGF signaling as wings, consistent with our model for the evolution of disproportionate or exaggerated weapon size from enhanced tissue-specific sensitivity to the insulin/IGF pathway.

A growing body of research now implicates insulin/IGF signaling in the development of extreme animal structures (23). Insulin/IGF signaling is an ancient and conserved physiological pathway that has coupled rates of cell proliferation with available nutrients for at least 500 million years, and we suggest that this pathway has been co-opted repeatedly in lineages experiencing strong sexual selection to yield disproportionate growth in signaling structures. The insulin/IGF pathway would likely have controlled the rate of growth of these structures already; increased cellular sensitivity to these signals would therefore be an easy route to the evolution of accelerated growth if the structure came under directional sexual selection for increased size.

But such a route to exaggeration would only generate exaggerated trait sizes in high-condition individuals because low-condition individuals would have low circulating concentrations of insulin/IGF signals and attenuated rates of tissue and body growth. The same mechanism stimulating increased trait growth in high-quality individuals would also repress trait growth in low-quality individuals (Fig. 1B). This means that whenever exaggerated ornament or weapon size arises due to an increase in trait-specific sensitivity to insulin/IGF signaling,

Fig. 2. Effect of insulin receptor (*InR*) knockdown on growth of adult structures in rhinoceros beetles. (A to C) Relative transcript abundances for the insulin receptor (*InR*) gene in genitalia (A), wings (B), and horns (C), measured 24, 48, 72, and 96 hours after the onset of the prepupal period in control (open bars) and dsRNA-injected (solid bars) animals. Injection with dsRNA significantly reduced transcript abundances for 48 hours after injection in all three tissues. (D to F) Effects of dsRNA knockdown on trait growth. Genitalia were insensitive (D); wings responded significantly but moderately to interrupted insulin/IGF signaling (E) (average reduction in wing length = 2%); and horns responded markedly (F), with an average reduction in horn length of 16%. (G) Head and thorax shown in two orientations (top and bottom) for same-sized control (left) and dsRNA-injected (right) males.



then the exaggerated trait should also show enhanced (or “heightened”) condition-sensitive expression and higher relative variability in trait size between low- and high-condition individuals (as compared to other, nonexaggerated, traits). Signal reliability would be an intrinsic property of these

structures because of the developmental mechanism regulating their growth. Theoretical considerations of sexual selection and animal signaling argue that escalated evolution of signals is most likely when signals are reliable, and it is difficult or impossible for low-

quality males to “cheat” by producing full-sized structures (Fig. 3). Signal reliability can be evolutionarily stable under two sets of conditions: Either the signal is sufficiently costly to produce or wield that it is not cost-effective for low-quality individuals to cheat (“handicap” signals),

Category of models:	Model conclusions:	Model predictions:	Contribution of the insulin/IGF mechanism:
Corroborated by an insulin/IGF mechanism for trait exaggeration:			
Index signal models Maynard Smith & Harper 2003 (3)	Sexually selected traits can evolve to exaggerated sizes if bigger versions of the trait are more effective signals than smaller versions, and if trait size is a physiologically or physically unfakable ‘index’ of the quality of the signaler. Trait size is a reliable signal because it is mechanistically impossible for low quality signalers to cheat.	Should be mechanistically impossible for low quality males to produce large signal traits. Why the size of a signal trait is unfakable is often unclear/unspecified.	Provides an explicit mechanism for unfakable signal expression in exaggerated morphological structures.
Good genes/indicator models Andersson 1986 (26) Iwasa & Pomiankowski 1999 (13) Lorch et al. 2003 (27)	Females benefit if they choose mates based on conditionally expressed ornaments because variation in the expression of these traits reliably indicates the overall quality of a male. Low quality males produce smaller signal traits because they are in poor condition.	Exaggerated traits should exhibit ‘heightened’ conditional expression.	Provides an explicit mechanism for heightened conditional expression of exaggerated morphological structures.
Genic capture models Rowe & Houle 1996 (12) Lorch et al. 2003 (27) Tomkins et al. 2004 (28)	Evolution of ornaments persists (genetic variation is not depleted) because in their expression these traits “capture” genetic variation for overall body condition, including health, resistance to parasites, competitive ability, nutrition, etc.	Genetic variation among males affecting their body condition, resistance to parasites, competitive ability, etc., should translate into differences in ornament size.	Provides an explicit mechanism for genic capture, since all of these aspects of body condition are channeled into a common endocrine signal regulating trait growth.
Assessment/arms race models Parker 1974 (29) Enquist & Leimar 1983 (30)	Male weapons can evolve to exaggerated sizes if weapon size reliably signals the fighting ability of a male.	Males should use relative weapon size as a basis for assessment; fights should be most likely to escalate if rival males are similarly armed. Not clear from the models why weapon size should remain reliable.	Suggests that weapons will become increasingly reliable signals of fighting ability as they increase in size, facilitating arms races.
Modified by an insulin/IGF mechanism for trait exaggeration:			
Handicap models Zahavi 1975 (24) Pomiankowski 1987 (31) Grafen 1990 (25) Johnstone 1995 (11) Iwasa & Pomiankowski 1999 (13)	Females benefit if they choose mates based on costly ornaments. A given increase in trait size costs low quality males more (or benefits them less) than it does high quality males, resulting in ornament sizes that reliably signal male quality.	Costs should be present, and they should be relatively highest for low quality males.	Suggests that costs may not be necessary for maintaining signal reliability*, and the handicap principle may only be relevant when exaggeration is extreme.
Allometry evolution models Bonduriansky & Day 2003 (32) Kodric-Brown et al. 2006 (33)	Exaggerated ornaments/ weapons will have steep allometry slopes when small males pay higher costs (or derive fewer benefits) than large males for the same increase in trait size.	Costs of ornaments/weapons should trade-off with allocation to overall growth or body maintenance, and these costs should be relatively highest for small males.	Suggests that costs may not be necessary for steep allometry slopes (they should arise as a byproduct of the mechanism of exaggeration)*. This should expand the conditions for which steep allometry slopes are expected.

*Costs will still work to ensure signal reliability in both cases (i.e., the models are not wrong); however, for insulin-sensitive exaggerated structures, costs may not be necessary. These structures should be intrinsically reliable due to the proximate mechanism regulating their growth.

Fig. 3. Sexual selection models whose relevance is affected by the proximate mechanism responsible for trait exaggeration.

or the signal is intrinsically unfakable (“index” signals, “good genes” signals) (2–4, 11–13, 24–33). The largest ornaments and weapons are generally assumed to be handicap signals of male quality, such that the cost of these structures enforces signal reliability (2–4, 24–33). However, for even the largest structures, the process of escalation must have started when these structures were small, and at that early stage, these costs would likely have been minimal. Moreover, several recent studies of exaggerated male ornaments and weapons have failed to find appreciable costs (34, 35), forcing a reconsideration of the question, why don’t low-quality males cheat?

We suggest that exaggerated animal structures may be unfakable signals of quality because of the developmental mechanism responsible for their accelerated growth. If true, then our hypothesis of “intrinsic reliability” could help explain why so many different signal traits embark on an evolutionary trajectory of bigger and bigger size. We suggest that whenever receivers responded to variation in insulin/IGF-sensitive structures, they fared relatively well due to the intrinsic reliability of these traits as signals of underlying male quality. As these traits became larger under selection, their utility as signals would have increased, enhancing the benefits to receivers and accelerating the rate of signal evolution still further. Once these structures become large enough to be costly, they may also act as handicap signals, and costs could contribute to signal reliability (Fig. 3). However, as long as the traits exhibit heightened sensitivity to insulin/IGF signals, costs may not be necessary for signal reliability (36). This means that subsequent evolution of compensatory structures alleviating costs to the signaling males (37) need not undermine the reliability of these traits as signals and could explain why some exaggerated sexually selected structures function as reliable signals even when no discernable costs are apparent (34, 35).

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Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1224286/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S3
Tables S1 and S2
References (38–90)

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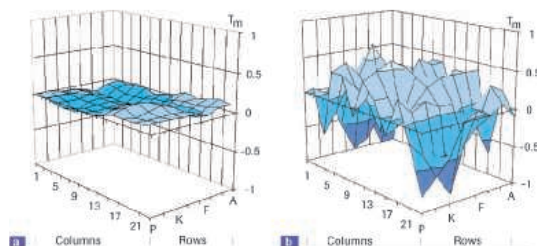


Figure 1: Influence of the Therma-Base layer on temperature homogeneity across a 384-well plate.
(a): LightCycler® 480 Real-Time PCR System including Therma-Base; **(b):** 384-well plate on Applied Biosystems 7900HT without Therma-Base.

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Minimum Qualifications: M.D./D.O. with board certification and eligibility for Texas medical license or Ph.D. in a field related to diabetes/obesity research from an accredited institution of higher education. Minimum 3 years of postdoctoral experience, and a strong publication record.

Preferred Qualifications: Candidates with experience in diabetes/obesity research and experience in using the latest technology preferred. Funded grant support in an area of basic, clinical, or translational research strongly preferred. Regional or emerging reputation in the area of diabetes and obesity research for consideration at the rank of Associate Professor. Interest in assisting with the development of a new Graduate School of Biomedical Sciences strongly preferred. Access to graduate students will depend on development of a successful training program, and we look to the candidates to participate in the development of the program. A willingness to start something new, and to help a new institution to grow and develop, is also a strongly desired characteristic. Good communication skills and demonstrated ability to work in a collegial environment are essential.

The Center will provide start up funds and partial salary support. 50% of salary should be supported by grant funding or a combination of grant funding and clinical/teaching work. Interested candidates must apply online at <http://jobs.texastech.edu>, requisition **84742**. A curriculum vitae, a short write up of research interests and three references may be attached and sent to: **Charles Miller, PhD, charles.miller@ttuhsc.edu, Chair, Department of Biomedical Sciences**. The position is open until filled. Application review will begin immediately.

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The **Department of Biological Sciences** is searching for candidates whose research focuses on the relation of epigenetics and chromatin biology to cancer initiation, progression, survival and metastasis. Additional areas of interest include regulation of self-renewal in cancer stem cells and epigenetic therapy of cancer.

Candidates of interest to the **Department of Horticulture and Landscape Architecture** are those who are studying molecular epigenetic mechanisms such as DNA methylation/demethylation and other modifications, chromatin remodeling and non-coding RNAs with focus on high-impact fundamental understanding of plant growth, development, evolution or adaptation.

The **Department of Nutrition Science** is searching for candidates conducting research on how environmental factors or metabolism interact with epigenetics. Departmental signature areas are bone health; dietary bioactives; ingestive behavior; metabolism and obesity; and cancer prevention. All research areas will be considered.

Candidates of interest to the **Department of Medicinal Chemistry and Molecular Pharmacology** include those whose research focuses on the relation of epigenetics and chromatin biology to drug discovery. Examples of areas of interest include, but are not limited to, (i) identification of epigenetic drug targets; (ii) development of chemical entities targeting epigenetic factors; (iii) characterization of epigenetic mechanisms underlying drug responses (pharmacoeugenetics); and (iv) development of new technologies in identification, detection and diagnostics of epigenetic/epigenomic markers for pharmacotherapy.

Finally, applications are sought from candidates conducting basic or applied research relevant to the scientific interests of **any department in the College of Agriculture** (<https://ag.purdue.edu/pages/departments.aspx>). Within this search, the departmental affiliation for the successful candidate will be selected by the incumbent.

Each successful candidate will be expected to develop an internationally recognized research program, interact with diverse faculty, staff and students across campus, contribute to the further development of epigenetics and chromatin biology as an area of excellence on the Purdue University campus, demonstrate excellence in teaching, and function as an active member of the departmental and university faculty.

Purdue University is a large and vibrant life science community. Our faculty spans disciplines that include biological sciences, physical and computational sciences, agriculture and engineering. Faculty also participate in interdisciplinary graduate programs with focus areas in cancer biology, plant biology, neuroscience, biophysics, gene regulation and bioinformatics. Support facilities are available for genomic analysis, metabolomics, protein mass spectrometry, NMR, X-ray crystallography, microscopy and confocal imaging, flow cytometry, and transgenic animal research.

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Applications and three letters of recommendation should be submitted electronically to ECB-search@purdue.edu. Applicants may indicate their top two choices of positions for which they would like to be considered. Screening of applications will begin **September 4, 2012** and will continue until the positions are filled. A background check will be required for employment in these positions.

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Applicants are requested to provide a cover letter, a curriculum vitae including list of publications, a statement of research accomplishments and future research plans, a description of teaching experience and aspirations, and the names and contact information of three persons whom we can contact for letters of recommendation. Review of applications will begin on **October 15, 2012** and continue until the position is filled. The appointment is anticipated to begin September 1, 2013.

Interested candidates should apply online at **AcademicJobsOnline.Org**. Questions should be directed to **Dr. Mark Denny at HopkinsSearch@lists.stanford.edu**.

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All applications should reach RIKEN by September 24th, 2012.

Applicants should address all correspondence to:

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Applications must include a curriculum vitae, a list of publications, and statements of future research and teaching plans. These materials, as well as three reference letters, must be submitted to our web-based application system using the following link:

<http://www.chemistry.fas.nyu.edu/object/chem.nyufacultypositions>

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- Cancer prevention and control/cancer epidemiology.

Applicants must have active extramural research funding, ideally from NCI, a strong track record of independent research, and ideally experience working in an NCI designated Cancer Center. Successful applicants will join a collaborative program that works closely to promote translational research with clinical research oncologists. Organizational strategic interests include the relationship between obesity, HPV and smoking as etiological risk factors for cancer. Applications in these research areas are especially welcomed.

A competitive salary and start-up package, to commensurate with experience and academic qualifications is available. Interested candidates may apply at <http://www.georgiahealth.edu/facultyjobs/> or a summary of research interests, curriculum vitae and names of three references should be sent to: **John Cowell, PhD, DSc, FRCPath, Acting Associate Cancer Center Director for Basic Science, Georgia Health Sciences University Cancer Center, Augusta, GA 30912-3125; E-mail: jcowell@georgiahealth.edu**.

The Georgia Health Sciences University is an Equal Opportunity Affirmative Action, and Equal Access Employer. The Georgia Health Sciences University has a strong interest in promoting diversity in its faculty and women and minority candidates are encouraged to apply.

FRED HUTCHINSON CANCER RESEARCH CENTER

A LIFE OF SCIENCE

The Division of Basic Sciences invites applications at the **junior faculty member** level. We are seeking outstanding scientists conducting cutting-edge research into fundamental biological questions. Current faculty members investigate diverse areas of genetic, molecular, cellular, metabolic, developmental, evolutionary, structural and computational biology and utilize a broad range of approaches and experimental systems. We support our junior faculty by providing a collegial environment and a wealth of technical resources. The Fred Hutchinson Cancer Research Center is located in a modern campus by Lake Union in Seattle, Washington, and is close to other non-profit research institutes and the University of Washington. It has an outstanding graduate program and state of the art facilities and research resources. Further information about the Center is available at <http://www.fhcrc.org/basic>.

Candidates should visit <http://www.fhcrc.org/basicfacesearch> for application instructions.

Application deadline: November 9, 2012

The Fred Hutchinson Cancer Research Center is an Equal Opportunity Employer, committed to workforce diversity.



UNIVERSITY of
ROCHESTER
MEDICAL CENTER

TENURE TRACK/TENURED POSITIONS CENTER FOR RNA BIOLOGY

The Center for RNA Biology, directed by **Lynne E. Maquat, PhD**, and co-directed by **David H. Mathews, MD, PhD**, is soliciting applications from outstanding individuals holding a PhD and/or an MD degree(s) and at least two years of post-doctoral training for a position at the **ASSISTANT, ASSOCIATE or FULL PROFESSOR** level. Emphasis is being placed on wet-bench and/or computational studies having disease relevance, including but not limited to the study of RNA metabolism or the use of RNA as a therapeutic tool or target. Successful applicants are expected to develop independent, externally funded research programs and to contribute toward graduate and medical-school teaching. The University of Rochester Medical Center and the adjacent undergraduate College of Arts & Sciences offer an outstanding research environment with established strengths in RNA Biology and excellent opportunities to collaborate with basic scientists and clinicians. Applicants should apply on line at www.rochester.edu/joboppp, job number 176618 and forward their CV, descriptions of research accomplishments and future research plans, and letters from three references to **Sharon Kubiak, Manager of Candidate Administration Services** at sharon_kubiak@urmc.rochester.edu

The University of Rochester has a strong commitment to principles of diversity and, in that spirit, actively encourages applications from groups underrepresented in higher education.

SRM UNIVERSITY

CHENNAI, INDIA

SRM University, is part of four decade old SRM Group of Educational Institutions. The University offers Undergraduate, Postgraduate, Doctoral and Post-Doctoral programs in Engineering, Architecture, Dentistry, Medicine, Allied Health Sciences and Science & Humanities. There are approximately 28000 students and 2400 Faculty and Staff members. SRM Engineering College, constituent of the SRM University, has been accredited by Indian National Board of Accreditation-AICTE-with an "A" grade. It is also an ISO 9002 certified institution. The Electronics & Communications Engineering program at SRM University has been accredited by USA based **Accreditation Board for Engineering and Technology, Inc. (ABET)**. The University is in the process of getting ABET accreditation for other engineering disciplines also.

The University through the SRM Research Foundation has fostered research in its various programs. The Faculty Members are highly encouraged to conduct research in their respective field. Outstanding research works are recognized by the University management and individuals are rewarded for their work.

SRM University is in the process of expanding the Faculty in every field of Engineering, Medicine, Management and Science. We are inviting applications from qualified candidates for the position of Professor, Associate Professor, Assistant Professor, Research Associate and Post-Doctoral Fellows. All these positions are open to International Academia, Indian and Non-Resident Indians (NRI). Suitable work visa will be arranged by the University wherever necessary. The selected candidates should be willing to relocate to Chennai, India preferably for at least 2-3 years. We also invite candidates who are on sabbatical, for teaching courses and conducting research for one semester. The prospective applicants may visit our website: www.srmuniv.ac.in.

Eligibility Criteria:

- **Professor:** Ph.D. with at least 10 years of academic/industry experience with demonstrated excellence in Research and Teaching.
- **Associate Professor:** Ph.D. with at least 5 years of academic/industry experience with demonstrated excellence in Research and Teaching.
- **Assistant Professor:** Ph.D. with at least 3 years of academic/industry experience with demonstrated excellence in Research and Teaching.
- **Research Associate:** Ph.D. with at least one year of demonstrated research experience.
- **Post-Doctoral Fellow:** Ph.D.

Compensation package will include competitive salary and full benefits (Medical, Dental, Paid Leave, Travel allowance etc.)

Interested candidates are requested to send their latest CV to: hrd@srmuniv.ac.in

Please include in the correspondence the salary expectation and the required lead time for joining.

The Registrar, SRM University, SRM Nagar, Kattankulathur – 603203. Kancheepuram Dist, Chennai Area. Tamil Nadu, India.

E-mail: hrd@srmuniv.ac.in

Ph: +91 44 2741 7000 / 2741 7777

www.srmuniv.ac.in



SRM

UNIVERSITY

(Under section 3 of UGC Act 1956)

ASSOCIATE OR FULL PROFESSOR

Department of Chemistry

ARTS AND SCIENCE

The Department of Chemistry at New York University invites applications for a tenured faculty appointment at the rank of Associate or Full Professor. All areas of chemistry will be considered, but applicants specializing in physical or inorganic chemistry will receive special consideration. The Department of Chemistry at NYU is implementing a significant growth plan, and the appointee will play an active role in this expansion. The successful applicant will be located in a newly renovated state-of-the-art multi-investigator laboratory, modeled after the recently established Biomedical Chemistry Institute and Molecular Design Institute. Duties will include undergraduate and graduate teaching. Candidates should have an established record of excellence in research and teaching. The anticipated start date is September 1, 2013, pending budgetary and administrative approval.

Applications must include a curriculum vitae, a list of publications, and statements of research and teaching plans. These materials, as well as three reference letters, must be submitted to our web-based application system using the following link:

<http://chemistry.fas.nyu.edu/object/chem.nyufacultypositions>

Application review will begin **September 17, 2012**. If you have any questions about this position, please send an email to chemistry.search@nyu.edu.



NEW YORK UNIVERSITY

NYU encourages applications from women and members of minority groups and is an Equal Opportunity/Affirmative Action Employer.



GEORGETOWN UNIVERSITY

Senior Faculty Position Theoretical or Computational Soft Condensed Matter Science

Georgetown University invites applications for a senior faculty position (Associate or Full Professor) in the field of computational and/or theoretical soft condensed matter. Applicants should have a broad, internationally recognized record of research accomplishments studying the properties of materials such as complex fluids, gels, emulsions, biomaterials, or active matter, and demonstrated excellence in teaching at the undergraduate and graduate level. The new hire will join the newly founded Institute for Soft Matter Synthesis and Metrology (<http://softmatter.georgetown.edu/>), a joint effort of the Departments of Physics and Chemistry. The mission of the Institute is to support research and education in the science of soft matter at Georgetown and in the broader community. Georgetown's commitment to the Institute includes at least two additional hires in soft matter in the near future. It is anticipated that the hire will actively participate in the growth of the Institute and collaborate with current Institute members.

Applicants should forward a CV, a concise statement of research and teaching plans, and contact information for at least four references via e-mail to softmatter@georgetown.edu or to **Professor Jeff Urbach, Director, ISMSM, Department of Physics, Georgetown University, Washington, DC 20057**. Review of applications will begin **September 15, 2012** and will continue until the position is filled.

Georgetown University is an Affirmative Action/Equal Opportunity Employer that is committed to diversity in the workplace.



南京大學

NANJING UNIVERSITY

"Deng-feng Scholar" Position: Nanjing University, China

Nanjing University, a key comprehensive university in China under the direct supervision of the Ministry of Education, dates from 1902 when it was known as Sanjiang Normal School. The university invites applications for "Deng-feng Scholar" position in the field of sciences, technology, medicine, social sciences, art and humanity. We particularly welcome creative and energetic individuals with strong backgrounds in all of those fields.

This position offers outstanding scientific resources, reasonable start-up funding, attractive yearly salary and living conditions. The position also offers the opportunity for young scholars to undertake research and studies at the international famous university.

For consideration please download the application form from http://rczp.nju.edu.cn/urp-publish/publish/XmlFile/1/W_88_313.html and submit filled form and the required attachments to relative school or department shown in the webpage. The application period is not limited. Questions regarding this position could also be directed to rcb@nju.edu.cn, tel. 86-25-83686783



Learning with Purpose

Careers with Mass Appeal

Department Chair for Biological Sciences

The University of Massachusetts Lowell Department of Biological Sciences invites applications for a full-time tenured faculty position at the rank of Full Professor to serve as Department Chair starting January 2013 or as soon as possible thereafter. We are seeking a distinguished mid-career individual with the vision and leadership to promote a rapidly growing department and to provide leadership for the further development of research and academic programs.

The Department has over 500 students, offers BS and MS degrees, and students can pursue PhD degrees through several interdisciplinary programs. Current faculty research interests include genetics, neurobiology, cancer biology, invertebrate biology, developmental biology, structural biology, virology, microbial ecology, evolution, and biogeochemistry. Our campus is located very near the vibrant academic and commercial biotechnology centers of Boston, Cambridge and Worcester.

Minimum Qualifications: Two years' experience in leadership; Five years' teaching at the undergraduate and graduate levels.

Preferred Qualifications: Significant record of funding and publication from a sustainable externally funded research program; Excellent communication and interpersonal skills; Ability to work with diverse student and faculty population.

To apply, visit <http://jobs.uml.edu>. Please submit a curriculum vitae, copies of several recent research publications, a statement of research and teaching interests, not to exceed three pages, and three letters of recommendation. Review of applications will begin September 30, 2012, and continue until the position is filled; however, the posting may close when an adequate number of qualified applications is received. Thank you for considering the University of Massachusetts Lowell as an employer of choice. We look forward to receiving your application.

The University of Massachusetts Lowell is committed to increasing diversity in its faculty, staff, and student populations, as well as curriculum and support programs, while promoting an inclusive environment. We seek candidates who can contribute to that goal and encourage you to apply and to identify your strengths in this area.



Strong Leader Required

icddr,b, an international public health research institution based in Bangladesh, has a 50-year history of innovation. Its research agenda reflects the full spectrum of global public health challenges affecting people living in poverty. In addition to world-class research, icddr,b provides vital humanitarian services, and has a strong record of knowledge translation, policy influence and training.

Executive Director

icddr,b seeks an Executive Director to lead the institution through an exciting period of transformation and continue the implementation of a strategic plan approved by its international Board of Trustees and supported by core donors.

Reporting to the Board, the Executive Director is responsible for the strategic leadership and management of the organisation by:

- Providing institutional vision and strategy
- Consolidating and developing strong relationships with external stakeholders
- Delivering effective institutional management, fiscal responsibility and monitoring and evaluation.

The successful candidate will be a recognised professional with academic credentials in a health-related discipline. The candidate will have the capacity to inspire, build and manage teams comprised of individuals with diverse backgrounds and interests in a multi-disciplinary institution. A decisive leadership style with a track record of monitoring performance, delivering results, building funding partnership is required and an ability to advocate with a range of domestic and international audiences is essential.

icddr,b offers an internationally competitive remuneration package paid in US dollars. The employment contracts are for an initial three-year term with the possibility of extension.

Interested applicants are invited to send a confidential letter of application summarising the relevance of their experience with a detailed curriculum vitae, including salary requirements and three referees with contact information to: executivedirectorsearch@icddr.org

For detailed job description and information about the institution please visit:

www.icddr.org/executivedirectorsearch or contact the Director, Human Resources at +88-02-988-2407.

Closing Date: 20 September 2012

icddr,b is an equal opportunity employer and particularly welcomes applications from women candidates.



EXECUTIVE VICE CHANCELLOR FOR RESEARCH Worcester, MA

The University of Massachusetts Medical School (UMMS) invites applications and nominations for the position of Executive Vice Chancellor for Research (EVCR). UMMS has grown into a highly productive and collaborative research enterprise with first-rate scientific resources and facilities and over \$277M of research awards annually. The Executive Vice Chancellor for Research will be expected to bring strategic vision and strong management skills to play a major leadership role in this exceptional public institution. The position requires a leader with a proven track record of excellence in research administration and operations, a rigorous research background, and outstanding communication skills.

Reporting to Dr. Terence Flotte, the Dean of the School of Medicine and Provost & Executive Deputy Chancellor of the Medical School, the Executive Vice Chancellor for Research will serve as a member of the senior executive team of UMMS. S/he will advocate for the research ambitions of the faculty and ensure that the infrastructure is in place for the highly talented staff to succeed. The Executive Vice Chancellor must continue to nurture an intellectual atmosphere that will attract and support world-class scientists and physicians, reinforce the excellence of UMMS's basic science research, and foster the translation of basic science into clinical excellence. S/he must understand how to support and enable excellent scientists and have a vision that will help guide the future direction of UMMS's research.

All inquiries, nominations/referrals and applications (resumes and cover letters) may be directed in confidence to: **Stephanie Fidel, Principal, John Muckle, Managing Associate, Ariannah Mirick, Associate, Isaacson, Miller, 263 Summer Street, 7th Floor, Boston, Massachusetts 02110, e-mail: 4584@imsearch.com.** Electronic submission of materials is preferred. Isaacson, Miller will continue to accept applications until the position is filled.

As an equal opportunity and affirmative action employer, UMMS recognizes the power of a diverse community and encourages applications from individuals with varied experiences, perspectives, and backgrounds.



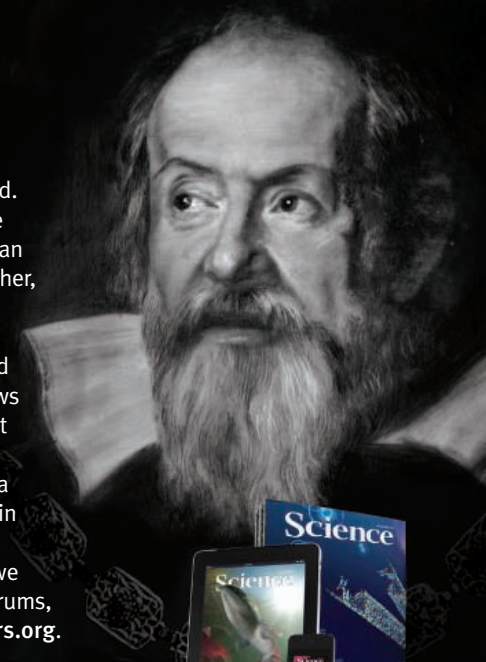
Science Careers

There's only one GALILEO GALILEI

Born in 1564, Galileo Galilei once contemplated a career in the priesthood. It's perhaps fortunate for science that upon the urging of his father, he instead decided to enroll at the University of Pisa. His career in science began with medicine and from there he subsequently went on to become a philosopher, physicist, mathematician, and astronomer, for which he is perhaps best known. His astronomical observations and subsequent improvements to telescopes built his reputation as a leading scientist of his time, but also led him to probe subject matter counter to prevailing dogma. His expressed views on the Earth's movement around the sun caused him to be declared suspect of heresy, which for some time led to a ban on the reprinting of his works.

Galileo's career changed science for all of us and he was without doubt a leading light in the scientific revolution, which is perhaps why Albert Einstein called him the father of modern science.

Want to challenge the status quo and make the Earth move? At *Science* we are here to help you in your own scientific career with expert career advice, forums, job postings, and more — all for free. Visit *Science* today at ScienceCareers.org.



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Career advice | Job postings | Job Alerts | Career Forum | Crafting resumes/CVs | Preparing for interviews



Harvard University Faculty of Arts and Sciences Department of Organismic and Evolutionary Biology TENURE-TRACK OR TENURED PROFESSOR

Position description: The Department of Organismic and Evolutionary Biology seeks to appoint a tenure-track or tenured professor in the field of vertebrate paleontology, emphasizing an evolutionary perspective. We seek an outstanding scientist who will establish an innovative research program and teach both undergraduate and graduate students. We are especially interested in individuals who conduct rigorous, field- and/or laboratory-based analyses of general problems in paleontology and evolution of vertebrate animals, and who employ morphological, functional, developmental, molecular and/or phylogenetic approaches. The successful candidate will receive a curatorial appointment in the Museum of Comparative Zoology (MCZ) with oversight responsibilities for the museum's vertebrate paleontology collection. The appointee will teach and advise at the undergraduate and graduate levels.

Basic qualifications: Ph.D. required by expected start date.

Additional qualifications: Demonstrated excellence in teaching and research is desired. Candidates for a tenured appointment should also evince intellectual leadership and impact on the field and potential for significant contributions to the department, University, and wider scholarly community.

Special instructions: Applications should include a cover letter, a curriculum vitae, statements of research and teaching interests, and representative publications. Review of applications will begin on **October 1, 2012**:

- Tenured faculty should submit these materials at <http://academicpositions.harvard.edu/postings/4228>
- Tenure-track applicants should submit these materials, along with the names and contact information of three to five references at <http://academicpositions.harvard.edu/postings/4227>

Letters of nomination from third parties are also welcome.

Contact information: Further information about OEB and MCZ are available at <http://www.oeb.harvard.edu> and <http://www.mcz.harvard.edu>.

Contact email: fac-search@oeb.harvard.edu

Harvard is an Equal Opportunity/Affirmative Action Employer. Applications from women and minorities are strongly encouraged.



Yale School of Forestry & Environmental Studies Junior or Senior Faculty Position in Tropical Forest Management

The Yale School of Forestry & Environmental Studies has an open rank, tenure-track opening for an exceptional natural scientist whose research focuses on the conservation and management of tropical forest resources. Ideal candidates will conduct interdisciplinary, field-based, hypothesis-driven research that bridges the social and biophysical sciences. They will also demonstrate potential for collaborating with natural, physical and social scientists at Yale and within a strongly interdisciplinary School. Areas of research could include: the science, policy and management of forest resources; biodiversity; climate-change related interventions; and the intersection between forest resource management, agriculture and land use. The ability to develop student field experiences, teach classes, and conduct research in both temperate and tropical systems would be an asset.

The successful candidate will be expected to develop an internationally recognized research program, and will teach introductory and advanced classes in the Yale School of Forestry & Environmental Studies and also in Yale College.

Applicants should submit a curriculum vitae, a statement of research and teaching interests, and the names of four references via email with the subject line "Environment Faculty Searches – Tropical Forest Management" to fesdeansoffice@yale.edu or via surface mail to: **Tropical Forest Management Faculty Search**, c/o Angela Kuhne, Dean's Office, Yale School of Forestry & Environmental Studies, 195 Prospect Street, New Haven, CT 06511, USA.

Prior to applying, candidates should explore the School's website (www.environment.yale.edu) and consider how their expertise can strengthen or complement the strengths of the existing faculty of the School. Applications received by **October 5, 2012** will receive full consideration. For more information about the position, contact Assistant Dean Angela Kuhne at angela.kuhne@yale.edu.

Yale University is an Affirmative Action/Equal Opportunity Employer. Men and women of diverse racial/ethnic backgrounds and cultures are encouraged to apply.

BCM[®]

Baylor College of Medicine

FACULTY POSITION DEPARTMENT OF PHARMACOLOGY

The Department of Pharmacology invites applications from outstanding scientists for a tenure track position at the Assistant, Associate or Full Professor rank. A competitive laboratory start up package will be provided to the successful candidate to support the development of an independent, funded research program in drug discovery, chemical biology, protein design and engineering, nanomedicine, pharmacogenomics or other areas broadly relevant to pharmacology. Candidates are also eligible for cross appointment in the Center for Drug Discovery. Candidates should have a Ph.D. and/or M.D. degree and postdoctoral experience as well as a strong record of research accomplishments. Baylor College of Medicine is located in the Texas Medical Center and offers a highly interactive environment and a strong infrastructure for research. Applications will be reviewed continuously until the position is filled.

Applicants should submit a statement of research interests and curriculum vitae as a single PDF to pharmacology@bcm.edu. Three letters of reference should be sent separately to pharmacology@bcm.edu, attention:

Timothy Palzkill, Ph.D.
Chair, Department of Pharmacology
Baylor College of Medicine
One Baylor Plaza
Houston, TX 77030

Baylor College of Medicine is an Equal Opportunity/Affirmative Action and Equal Access Employer.

WOMEN IN SCIENCE

forging new pathways in green science

Read inspiring stories of women working in "Green Science" who are blending a unique combination of enthusiasm for science and concern for others to make the world a better place.



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ScienceCareers.org/LOrealWiS



This booklet is brought to you by the AAAS/Science Business Office in partnership with the L'Oréal Foundation



Faculty Positions in Continental Margins and Coastal Environments

Scripps Institution of Oceanography (SIO) at UC San Diego (<http://sio.ucsd.edu>) is committed to academic excellence and diversity within the faculty, staff, and student body. SIO is a world renowned center of marine research with approximately 200 principal investigators leading research programs on all aspects of earth, ocean and atmospheric sciences.

Coastal Processes #10-439: We seek outstanding candidates with complementary interests in the physical oceanography of the coastal environment. Topics of interest are focused on the shelf, inner shelf, and near shore, including tidal inlets, estuaries, river mouths, beaches, and headlands. Some examples of physical interest are: surf zone processes; surf zone-inner shelf exchange; sediment, chemical, and biological transport; waves (internal and surface); and coastal response to sea level changes or human activity.

Coastal Ecology #10-440: We seek outstanding candidates with expertise in the biology and ecology of the coastal environment. Areas of interest broadly include the interactions of marine organisms with one another and with their physical and chemical environment. Research topics may include the ecology of benthic and pelagic organisms, ecological processes and regulatory mechanisms, population recruitment and connectivity, response to habitat changes and disruptions, bio(geo)chemistry and genomic adaptation. Research programs that integrate observational, experimental and modeling approaches in the field and laboratory are encouraged.

Geological, Geophysical and Geochemical Studies of Continental Margins #10-441: We seek outstanding candidates from throughout the Earth sciences with complementary skills targeting continental margin development, evolution, and physical/chemical exchange across a variety of spatial and temporal scales. An emphasis on the marine portion of the system is envisioned, but candidates investigating onshore aspects of margin processes that cross the shoreline will also be considered. We encourage applicants whose research bears on key processes at margins, including geochemistry, geophysics, dynamics, seismology, tectonics, structural geology, geodesy, petrology, geochemical exchange (high- and low-temperature), geomorphology, glaciology, and ice-margin studies.

All successful candidates will be expected to teach classes, supervise research at both the graduate and undergraduate level and contribute to leadership on issues of equity and diversity. The positions require a PhD degree and a competitive record of publication, as well as evidence of the ability to conduct and fund an active research program consistent with the opportunity to have done so at this career level. The successful candidates will also have demonstrated the highest standards of scholarship and professional activity, or for junior scholars to have the potential thereof. The department is interested in candidates who have demonstrated commitment to excellence by providing leadership in teaching, research or service towards building an equitable and diverse scholarly environment.

Our preference is for hiring at the level of assistant professor for all positions, however appointments at all ranks may also be considered. Salary will depend on the experience of the successful applicant and will be based on the UCSD pay scales. Completed applications received by **October 15, 2012** will be assured of consideration. Each position requires separate submission. All applications and related materials must be submitted electronically via Academic Personnel On-Line RECRUIT at: (<https://apol-recruit.ucsd.edu>). Emailed applications will not be accepted. Applicants should provide a current CV, cover letter including descriptions of their teaching experience, research interests, state if authorized to work in the U.S., and the names of three potential referees, along with their complete institution address, email address, phone and fax numbers, and a separate personal statement summarizing past or potential contributions to diversity (see <http://facultyequity.ucsd.edu/Faculty-Applicant-C2D-Info.asp> and <http://sio.ucsd.edu/Diversity/> for further information). Questions about submission of applications may be addressed to **Leslie Costi, (lcosti@ucsd.edu)**.

UCSD is an Affirmative Action/Equal Opportunity Employer with a strong institutional commitment to excellence through diversity.



Why not change the world?

School of Science at Rensselaer Polytechnic Institute **FACULTY EXPANSION INITIATIVE**

The School of Science at Rensselaer Polytechnic Institute in Troy, NY has a rich history of graduating highly influential and impactful scientists, researchers, scholars, and engineers. In 2011, President Shirley Ann Jackson, Ph.D. appointed Dr. Laurie Leshin, former senior executive and scientist at NASA, to lead the School of Science in significant growth that will solidify Rensselaer's reputation for excellence in scientific education and research, and to propel the School of Science forward in critical areas of national importance, need, and opportunity.

To achieve this historic growth, Dean Leshin is pleased to invite applications for faculty openings at all levels. Recruitment for this bold faculty expansion initiative in the School of Science is now underway, and faculty in Science Themes identified as strategic priorities and opportunities for the School and the broader Institute are needed immediately.

Candidates for all levels, including tenure-track Assistant Professor, Associate Professor and full Professor with tenure, are needed in areas that support the Science Themes as described below. Extraordinary candidates with highly visible, international reputations may be considered for appointment to a variety of endowed chair appointments.

More information on specific openings, required qualifications and application instructions, can be found at the School of Science website at <http://science.rpi.edu/>, and follow the link to "faculty searches". We encourage frequent visits to the School of Science homepage, as new information and additional postings will be added as they become available. Individuals wishing to be considered for one of these opportunities must follow the position specific application instructions found within each posting.

Origins & Fundamentals of Life & Matter

- Astrobiology (Physics, Applied Physics & Astronomy, Chemistry & Chemical Biology, or Earth & Environmental Science)
- Rayleigh Chair, Theoretical condensed matter physics, quantum optics, transport and photonics (Physics, Applied Physics & Astronomy)

Water, Energy & Sustainability

- D. Darrin Chair, Water (Biology or Earth & Environmental Science)
- Director of the Baruch Center for Biochemical Solar Energy (Chemistry & Chemical Biology)
- M. Darrin Chair, Computational math & modeling (Mathematical Sciences)
- Environmental geophysics (Earth & Environmental Science)
- Environmental informatics/simulation (Earth & Environmental Science)

Network & Data Science, Security & Visualization

- Hamilton Career Development Chair, cyber-risk (Computer Science)
- Multi-Agent systems (Computer Science)
- Constellation Chair in Biocomputation and Bioinformatics (Dept. open)
- Mobile computing, distributed systems (Computer Science)
- Environmental informatics/simulation (Earth & Environmental Science)

Biomedical Science & Applications

- D'Ambra Chair in Synthetic Organic and Medicinal Chemistry (Chemistry & Chemical Biology)
- Constellation Chair in Biocatalysis and Metabolic Engineering (Dept. open)
- Constellation Chair in Biocomputation and Bioinformatics (Dept. open)
- Constellation Chairs in Tissue Engineering and Regenerative Medicine (Dept. open)

Materials at the Nanoscale

- Rayleigh Chair, Theoretical condensed matter physics, quantum optics, transport and photonics (Physics, Applied Physics & Astronomy)
- Constellation Chairs in Tissue Engineering and Regenerative Medicine (Dept. open)

Modeling, Analysis & Simulation

- Ricketts Chair and Department Head (Mathematical Sciences)
- M. Darrin Chair, Computational math & modeling (Mathematical Sciences)
- Inverse problems/multi-scale modeling and simulation (Mathematical Sciences)
- Rayleigh Chair, Theoretical condensed matter physics, quantum optics, transport and photonics (Physics, Applied Physics & Astronomy)
- Environmental informatics/simulation (Earth & Environmental Science)
- Constellation Chair in Biocomputation and Bioinformatics (Dept. open)

Founded in 1824, Rensselaer Polytechnic Institute is the nation's oldest technological university. Institute programs serve undergraduates, graduate students, and working professionals around the world. Rensselaer offers more than 145 programs at the bachelor's, master's, and doctoral levels. Students are encouraged to work in interdisciplinary programs that allow them to combine scholarly work from several departments or schools. The university provides rigorous, engaging, interactive learning environments and campus-wide opportunities for leadership, collaboration, and creativity.



Rensselaer

We welcome candidates who will bring diverse intellectual, geographical, gender and ethnic perspectives to Rensselaer's work and campus communities. Rensselaer Polytechnic Institute is an Affirmative Action/Equal Opportunity Employer.

POSITIONS OPEN

FACULTY POSITIONS **Arizona Center on Aging** **The University of Arizona**

Applicants are invited for Tenured/Tenure-Track positions at the **ASSISTANT, ASSOCIATE**, and full **PROFESSOR** rank, depending upon qualifications, at the Arizona Center on Aging, University of Arizona College of Medicine, with a cross-appointment in one of the basic or clinical departments at the University of Arizona, depending upon the area of research expertise. Qualified applicants will develop strong, extramurally funded programs. Preference will be given to those candidates investigating how inflammation and innate immunity impact the process of aging and what are the sources and mechanisms behind increased proinflammatory state in older age; applicants with outstanding programs in other areas of biology of aging related to inflammation, such as endocrine aging, autophagy, sarcopenia or frailty research will be also considered. Successful applicants are expected to not only develop independent research programs and contribute to graduate (Ph.D.) and medical (M.D.) education, but also to invest a fraction of their effort to help build interactive and collaborative programs within and outside the Center to tackle larger biomedical problems relevant to human aging and age-related diseases.

The University of Arizona is amongst the top 20 public research and education universities, boasting excellent core facilities, strong departments and centers, as well as lively campus culture and life, and a blossoming recreations center. Located in sunny Tucson, a city with a vibrant multicultural population of approximately 900,000, the University has strong business, academic, and community partnerships. Tucson is surrounded by a majestic desert and mountains rising to more than 9,000 feet, and offers supreme outdoor recreational opportunities.

Please complete an online application for **Job #48568** at **website: <http://www.hr.arizona.edu>**. Be prepared to attach your curriculum vitae.

The University of Arizona is an Equal Employment Opportunity/Affirmative Action Employer. Minorities/Women/Persons with Disabilities/Veterans.

DUKE UNIVERSITY **Durham, North Carolina**

The Department of Chemistry invites applications and nominations for two tenure-track positions at the **ASSISTANT PROFESSOR** level to begin July 1, 2013. In combination with a commitment to teaching, candidates should have research interests in organic chemistry and/or the interface of chemistry and biology; we are particularly interested in candidates who would interface with ongoing university initiatives in molecular medicine, neuroscience, energy, or molecular imaging. Applications received by November 1 will be guaranteed consideration, although extraordinary applicants will be considered after this date. To guarantee consideration, all applicants should provide curriculum vitae, list of publications and description of research interests in PDF format to **e-mail: positions@chem.duke.edu**. Applicants should also arrange to have three letters of recommendation submitted on their behalf electronically to **e-mail: positions@chem.duke.edu**. *Duke University prohibits discrimination and harassment, and provides Equal Employment Opportunity without regard to race, color, religion, national origin, disability, veteran status, sexual orientation, gender identity, sex or age. Duke is committed to recruiting, hiring, and promoting qualified minorities, women, individuals with disabilities, and veterans.*

PLANT MOLECULAR BIOLOGIST

Tenure-track **ASSISTANT PROFESSOR** to begin September 2013; Ph.D. required. Eckerd College, Saint Petersburg, Florida. Teach seven course equivalents per academic year, including botany, genetics, cell biology, ecology, and evolution, other courses in the biology major, and January Term; participate in the general education program; develop a research program that includes undergraduates. Application deadline 21 September 2012 Full details **website: <http://www.eckerd.edu/hr/employment.php>**.